RESPONSES OF VARIOUS CARBON SOURCES FOR *in vitro* REGENERATION OF BANANA cv. CHINICHAMPA (AAB)

Thesis

Submitted to

NAGALAND UNIVERSITY

In partial fulfilment of requirements for the Degree

of

Doctor of Philosophy

in

Horticulture (Fruit Science)

by

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2024

Affectionately Dedicated

to my

Loving Family

DECLARATION

I, S. Kikatemjen Ozukum, hereby declare that the subject matter of this thesis is the record of work done by me, that the contents of this thesis did not form the basis of the award of any previous degree to me or to the best of my knowledge to anybody else, and that the thesis had not been submitted by me for any research degree in any other University/Institute.

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This is to certify that the thesis entitled **"Responses of various carbon sources for *in vitro* regeneration of banana cv. Chinichampa (AAB)"** submitted to Nagaland University in partial fulfilment of the requirements for the award of degree of Doctor of Philosophy in Horticulture (Fruit Science) is the record of research work carried out by Mr. S. Kikatemjen Ozukum, Registration No. Ph.D./HOR/00239 under my personal supervision and guidance.

The result of the investigation reported in the thesis has not been submitted for any other degree or diploma. The assistance of all kinds received by the student has been duly acknowledged.

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This is to certify that the thesis entitled **"Responses of various carbon sources for *in vitro* regeneration of banana cv. Chinichampa (AAB)"** submitted by S. Kikatemjen Ozukum, Admission No. Ph-269/18, Registration No. Ph.D./HOR/00239 to the NAGALAND UNIVERSITY in partial fulfilment of the requirements for the award of degree of Doctor of Philosophy in Horticulture (Fruit Science) has been examined by the Advisory Board on …….....

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ACKNOWLEDGEMENTS

First and foremost, I gave all my thanks to Almighty God for His blessing bestowed on me throughout this research programme.

With immense pleasure, I take this opportunity to express my heartfelt gratitude and profound appreciation to my advisor, Prof. C. S. Maiti for his constant guidance and encouragement throughout my research works without which this thesis would never have been successful.

My sincere thanks to all my Advisory committee members, Prof. Pauline Alila, Dr. A. Sarkar, Dr. L. Hemanta, Department of Horticulture and Dr. Pankaj Shah, Department of Genetics and Plant Breeding for providing necessary guidance and support in the course of my entire research work.

My special thanks also go to all the non-teaching staff of Department of Horticulture, led by Mr. Solo, Senior Technical Assistant, for the constant help rendered throughout my research. To the staffs of library for the facilities they rendered whenever required.

I express my overwhelming gratitude to all my Ph.D classmates, Khamrang Mathukmi, M. Michaerl Ariina, Ramit Konjengbam, John Debbarma, Lian, Jangyu and Omekali for their joyful company, timely help and cooperation during the entire course of my Ph.D study. Special thanks goes to Dr. Khamrang Mathukmi & Damitre Lytan for helping me in publishing & statistical analysis of my research works and thesis. Thank you all.

Lastly, I sincerely thank my beloved wife Dziesekhou, parents, siblings and my kids, Meyisenla, Maongtoshi and Modesty as well as well wishers for their unceasing prayers and love throughout the study period.

Date:

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LIST OF ABBREVIATIONS

%	:	Per cent
@	:	At the rate of
^{o}C	:	Degree centigrade
2, 4- D	:	2,4- Dichlorophenoxyacetic acid
AA	:	Ascorbic acid
ABA	:	Abscissic acid
AC	:	Activated charcoal
AD	:	Anno Domini
Ads	:	Adenine sulphate
ANOVA	:	Analysis of variance
BA	:	Benzyladenine
BAP	:	6- Benzylaminopurine
CD	:	Critical difference
CM	:	Coconut milk
cm	:	Centimetre
cv	:	Cultivar
DAP	:	Days after planting
DMRT	:	Duncan Multiple Range Test
et al.	:	Co-workers
g	:	Gram
GA_3	:	Giberellic acid
g/l	:	Gram per litre
ha	:	Hectare
$HgCl_2$	:	Mercuric chloride
IAA	:	Indole-3-acetic acid
IBA	:	Indole-3-butyric acid
iP	:	isopentyl adenine

i.e.	:	That is
kg	:	Kilogram
kn	:	Kinetin
MFBs	:	Male floral buds
mg	:	Milligram
mg/l	:	Milligram per litre
mm	:	Millimetre
mm^2	:	Millimeter square
MS	:	Murashige and Skoog
MT	:	Metric Tonne
NAA	:	α- Naphthalene acetic acid
ppm	:	Parts per million
RIM	:	Root Induction Medium
SEM	:	Standard error mean
SPM	:	Shoot Proliferation Medium
spp.	:	Species
TDZ	:	Thidiazuron
t/ha	:	Tonne per hactare
viz.	:	Namely
v/v	:	Volume by volume

ABSTRACT

The present research work entitled "Responses of various carbon sources for *in vitro* regeneration of banana cv. Chinichampa (AAB)" was conducted during the year 2020-2022 at Tissue culture Lab, NU:SAS. Two source of explant (suckers & immature male floral buds) were used in the present studies. Completely Randomized Design (CRD) with 3 replications were carried out for each treatments while the effects of treatments were tested by ANOVA and differences among the treatment means were tested using DMRT (Duncan, 1955) at 5% F-test level of probability.

The purpose of this investigation was to work out the best carbon sources for *in vitro* regeneration of banana cv. Chinichampa (AAB) through shoot tip and MFBs. Eight (8) different carbon sources *viz.* sucrose, glucose, fructose, mannitol, maltose, sorbitol, table sugar and jaggery were investigated in different detailed experiments. All together 7 parameters under each explants (suckers & male floral buds) were tested for *in vitro* culture establishment and shoot proliferation in two separate experiments. And all together 6 parameters under each explants (uniform multiple shoots from suckers and male floral buds) were tested for *in vitro* rooting establishment. Subculture studies up to 5^{th} subculture were also carried out for both the explants under different experiment. Primary and secondary acclimatization was done with different substrate under another experiment. Finally, differential cost analysis for production of 1000 plantlets was worked out and results were depicted in tabular format.

In the experiment, for *in vitro* culture establishment and shoot proliferation through shoot tip, the experimental result revealed that the minimum days to greening (4.63), minimum days for multiple buds initiation (24.28) and maximum length of multiple buds (3.03 cm) was observed in shoot proliferation medium (T_1 SPM) supplemented with 30 g/l sucrose in the

modified MS medium. The highest number of multiple buds (4.00) was recorded in shoot proliferation medium (T_{18} SPM) supplemented with 30 g/l table sugar.

For *in vitro* rooting through shoot tip, the minimum days (6.48) required for root initiation were observed in root induction media (T_3 RIM) supplemented with 30 g/l glucose. High rooting percentage (%) was recorded in media supplemented with 30g/l sucrose (96.67%) and table sugar (96.67-100%). Number of roots per shoot (4.59) was found highest in (T_{18} RIM) supplemented with 30g/l table sugar. Length of longest root (7.74 cm) after 30 days was found in (T_{17} RIM) supplemented with 20g/l table sugar. The highest number of leaves per plantlets (4.56) was observed in (T_1 RIM) supplemented with 30g/l sucrose.

In the experiment for *in vitro* culture establishment, shoot proliferation and rooting through male floral buds, the present investigation showed that the minimum days to greening (7.95), minimum days required for multiple buds initiation (44.67) and maximum number of multiple buds produced per explants (8.33) was observed in shoot proliferation medium (T_{18} SPM) supplemented with 30 g/l table sugar. Maximum length of multiple buds (3.01 cm) after 30 days was recorded in T_1 SPM with sucrose 30 g/l. Similarly, minimum days to root initiation (7.60) and rooting percentage (100.00%) were observed in root induction medium (T_{18} RIM) that contain 30 g/l table sugar. While the highest number of roots per shoot (4.87) and highest number of leaves per plantlets (5.08) were revealed in (T_1 RIM) with 30g/l sucrose.

Sub culturing was successfully repeated at 4 weeks interval up to 5th subculture and the maximum number of multiple buds (3.71 and 8.92) was recorded during the 3rd subculture in T_{18} SPM with table sugar 30 g/l in both the explants under experiment. Mean survival percent of *in vitro* plantlets during primary hardening was highest at 90% under coco peat:perlite (1:1)

combination. While the mean survival percent during secondary hardening was observed at 98% under sand:soil:FYM (1:1:1).

From the present investigation it is evident that there is higher potential in regeneration on the number of multiple buds using male floral buds (8.33) instead of regular shoot tips (4.00). However, days required for multiple buds initiation was found much earlier in shoot tip culture (24.28) as compared through MFBs culture (44.67). There was no significant difference in the rooting % established either through shoot tip or MFBs when cultured in half strength MS rooting medium. Therefore, we can summarized that, table sugar 30g/l as carbon sources gave the best performance for *in vitro* shoot establishment, proliferation and rooting of banana cv. Chinichampa (AAB) for both explants *viz*. shoot tip and male floral buds (MFBs) under study. While subculture can be successfully obtained up to 5^{th} subculture without any contaminations and subsequent acclimatization of primary and secondary plantlets were achieved at 90% and 98% respectively. There was 23.73 % cost reduction by replacing laboratory - grade sucrose with commercially available table sugar in the present study.

Through these studies we can suggest that there is a very high potential for banana *in vitro* studies on different cheaper carbon sources available to reduce the plantlet cost acceptable for commercialization of tissue culture industry/laboratory. More research works on banana *in vitro* using male floral buds (MFBs) is the need of the hour and future way ahead for producing disease free tissue culture banana plantlets to meet the growing farmers demand, because of its lesser contamination chances from explants as compared to suckers (shoot tips) and also more propagation ration can be achieved within the same cost and time.

Key words: Banana, carbon sources, *in vitro*, sucrose, table sugar.

CHAPTER I
INTRODUCTION

INTRODUCTION

Banana belongs to the genus *Musa* and family *Musaceae*. Banana (*Musa* spp.) is a monocotyledonous, herbaceous, perennial succulent plant and one of the most important edible food crops worldwide. It is known as one of the most important sources of tropical fruits in the world market, significant staple food and a major export commodity (Rahman *et al*., 2013). As a diet, it is highly satisfying, easy to digest, nearly fat free, rich source of carbohydrate with calorific value of 67g/100g and is free from sodium making it a salt free diet suitable to all the age groups and people of all levels. It contains various vitamins and has therapeutic values for the treatment of many diseases (Singh, 2007).

Bananas are predominantly produced in Asia, Latin America and Africa. Presently, banana growing in 150 countries across the globe on total area of 5.14 million ha and producing 105.32 million MT (Kumar, 2017). India is first in area and production of banana in the world. Its annual total production is around 30.47 million MT from about 86 lakh hectare area with average of 37.0 MT per hectare (Anon., 2018).

In India maximum production is in Andhra Pradesh (5003.07 000' MT) followed by Gujarat (4472.32 000' MT) and Maharashtra (4209.27 000' MT). The major banana growing states are Andhra Pradesh, Maharashtra, Assam, Bihar, Gujarat, Karnataka, Kerala, Madhya Pradesh, Orissa and West Bengal (Anon., 2018). Variation in productivity ranges from 13.5 to 63.6 MT/ha, which is attributed to cultivars, production system and management strategies. In North East Region of India, the production of banana is 1492 000' MT from an area of 1.0071 lakh with a productivity of 11.32 MT/ha, out of which production in Nagaland is around 117.04 000' MT from an area of 0.0834 lakh with a productivity of 14.03 tonnes / ha. Assam leads in both area (0.53308

lakh) and production (913.27 000’ MT) followed by Mizoram with an area of 0.1101 lakh and production of 117.04 000’ MT. Lowest production has been found to be in the state of Sikkim (3.71 000’ MT) (Anon., 2018).

In vitro propagated plants are steadily becoming the planting material of choice because of disease free, uniformity and the possibility of rapid multiplication. The yields and returns are expectedly higher (Hussein, 2012). Presently, there are about 200 commercial tissue culture companies in India with production capacity of around 500 million plantlets annually. The plant *in vitro* tissue culture market in India is estimated at ₹500 crores (Anon., 2016).

Although there is a significant commercial value of the banana, its main production constraint is the readily available of quality planting materials. The planting materials through suckers from fields do not meet the increasing demands and are of poor qualities. Commercial tissue culture techniques can solve these problems (Sugandh, 2017). Tissue culture techniques are becoming increasingly popular as alternative means for vegetative propagation in many plant species. Tissue culture also plays a pivotal role in germplasm exchange programmes, *in vitro* conservation, and rapid multiplication of new varieties and hybrids hastening the process of selection and breeding by plant breeders. Some disadvantages of micropropagation are that it requires cultivar specific standardizations for successful commercial adoption and significant presence of genetically modified plants due to somaclonal abberations (Saraswathi *et al*., 2014). The commercialization of *in vitro* propagation is mostly experimented in the varieties of Cavendish group (AAA). Factors responsible for success of *in vitro* commercialization are explant type and size, sterilization techniques adopted, media compositions, culture conditioning, acclimatization process etc. (Waman and Bhora, 2018).

In living plants and tissues, carbohydrates plays a crucial role as a main source of carbon in biosynthesis processes. In *in vitro* cell, tissue, and organ

cultures of plants which are not fully autotrophic, they require a constant source of carbohydrates to balance the osmotic potential of the media (Cuenca and Vieitez 2000; Huh *et al*., 2016). It also require a constant supply of energy and carbon for morphological processes with high energy requirement, such as caulogenesis, root initiation, embryogenesis, and shoot bud multiplication and elongation (Yaseen *et al*., 2013).

Carbohydrate has varying effects on growth development, synthesis of cells, and its physiology (Gibson, 2000). Sugar is also a very essential component in medium and its addition is essential for *in vitro* growth and development of plants because photosynthesis is insufficient, due to its weak development of their leaves, the limited gas exchange and the high relative humidity (Pierik, 1988; Kozai, 1991; Mazinga *et al*., 2014). Organized tissues show a better growth and proliferation after the addition in medium an adequate source of carbon (Mazinga *et al*., 2014). Sugars enter the metabolism pathways and transformation of energy which are required for growth of cell (Gauchan, 2012). *In vitro* micropropagation of plant is largely determined by the composition of nutrient medium. Carbohydrate strongly affects the growth and morphogenesis of *in vitro* cultured plant as it acts as a carbon source and osmoticum both (Lipavska and Konradova, 2004).

Sucrose is a non-reducing sugar and these types of sugars are less reactive than the reducing sugars (Carvalho *et al*., 2013). Therefore, non-reducing sugars are more translocated (Taiz and Zeiger, 2004). On the other hand, glucose, which is a reducing sugar, can be readily taken up providing a better carbon source, by diffusion across the cut tissues surfaces and metabolized by *in vitro* tissue culture (Welander and Pawlicki, 1994). In broad range sucrose is widely used as a foremost carbon in the medium for *in vitro* plant culture, since in the phloem sap of most plants sucrose is the common carbohydrate found **(**Fuentes *et al*., 2000; Lemos and Baker, 1998; Saraswathi *et al*., 2016). However, Landford and Wainwright (1988) reported that high

concentration of sucrose cause to reduce the photosynthetic activity and cell growth rate. However, according to many researchers, sucrose is not often the first choice carbon to achieve shoot multiplication (Blanc *et al*., 1999) because other carbon sources besides sucrose also are translocated in different plants (Moing *et al*., 1992).

Glucose and fructose are also known to hold up for growth of some tissues (Morfeine, 2014; Bhojwani and Rajdan, 1996). Many researchers have studied the effects of different carbon sources (*viz*., sucrose, glucose, fructose) and their concentrations on *in vitro* shoot multiplications and rooting of *Musa* (Madhulatha *et al*., 2006; Buah *et al.* 2000; Ilczuk *et al*., 2013; Brearley *et al*., 2014; and Morfeine, 2014). Different type, composition and dose of carbon sources had considerable effects on *in vitro* rooting and shoot multiplication of *Musa*, (Bohra *et al*., 2016; Qamar *et al.*, 2015; Ahmed *et al.,* 2014b; Waman *et al*., 2014; and Vora and Jasrai, 2011). According to Hossain *et al*., 2009, Glucose resulted in the highest production in callus induction and cell growth. Three per cent glucose was optimal in media for callus induction and cell growth. In the regeneration medium sucrose and sorbitol acts differently in embryo regeneration and they showed the highest and lowest number of embryos respectively.

Many researchers (Saraswathi *et al*., 2016; Prabhuling and Sathyanarayana, 2017; Placide *et al*., 2012; Agrawal *et al*., 2010) worked on different cost- effective and in expensive and readily available carbon sources of banana *in vitro* propagation. They found market sugar or table sugar, rock sugar, common grade sugar had no significant effects on shoot and root generation. Analytical grade sucrose contributes to the higher costs of media (34 per cent of the production cost) (Demo, 2008). There was 85 per cent decrease in cost of media for plant caulogenesis by using inexpensive carbon source, water source and gelling agents (Sharma *et al.,* 2013). According to Prakash, 1993, use of common sugar in place of laboratory grade sucrose reduce the cost of carbon

source of the medium by 78-87 per cent and AR grade sucrose by rock sugar by 95.85 per cent of sucrose cost (Prabhuling, 2010).

In Nagaland diverse banana species are found depending on the agro ecological situation of the state. However, only few cultivars are cultivated at commercial scale. Chinichampa (AAB) is a favourite among the different cultivars found. It is tall, vigorous and hardy. The distinguishing characters are the rose pink colouration of the outer side of the mid rib when young; heavy bunches with closely packed fingers hanging down vertically. It has good scope for commercialization in the state. It is resistant to panama wilt and tolerant to bunchy top disease but highly susceptible to banana bract mosaic virus and banana streak virus. (Kumar, 2012; Radha and Mathew, 2007). There is report on incidence of new strains of virus (BBrMV and CMV) spreading into NE region which were previously confined only in the South India and was not reported from NER of India (Selvarajan and Balasubramanian, 2017; Lepcha *et al*., 2017).

The sugar concentration favoured is also very essential for *in vitro* establishment of plantlets. In general, with increased sugar concentration, the growth and development increases, until an optimum is reached and then decreases at higher concentrations (De Paiva Neto and Otoni, 2003). Also, the effect of carbohydrates on the micropropagation can differ according to the species (Baskaran and Jayabalan, 2005). Sugars or carbon source are signaling molecules repressing or activating plant genes involved in many important functions such as photosynthesis, glycoxylate metabolism, respiration, starch and sucrose synthesis and degradation, nitrogen metabolism and cell cycle regulation (Jang *et al*., 1997 and Xiao *et al*., 2000). However sucrose has been identified as a key signaling molecule in plant development, including in tissue culture (Horacio and Martinez-Noel, 2013). Also sugars have a great potential effect on the physiology, growth and differentiation of cells and directly influence in shoot and root proliferation potential of *in vitro* explants Gibson

(2000). Thus, the carbohydrate requirements must be defined and optimized for each micropropagation system. Moreover there is no reports showing the success of *in vitro* propagation of *Musa* cv Chinichampa (AAB) using immature male floral buds.

Keeping in mind of all the above scenario, the present work was planned to study the responses of various carbon sources for *in vitro* regeneration of banana cv. Chinichampa (AAB), with the following objectives.

i. To study the responses of various carbon sources on *in vitro* culture establishment and shoot proliferation of banana explants cv. Chinichampa (AAB) through shoot tips and immature male floral buds.

ii. To study the responses of various carbon sources on *in vitro* rooting of banana cv. Chinichampa (AAB) through shoot tips and immature male floral buds.

CHAPTER II

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Some review of literature aimed at highlighting the previous worked carried out by researchers across the world on various aspects of Banana micropropagation, which are given below in following sub headings and will be referred thoroughly in the present research work.

2.1 Studies on *in vitro* culture establishment

Wong (1986) conducted an experiment using banana explants with apical domes and a total of 22 banana cultivars were successfully cultured on a modified Murashige and Skoog's medium containing 6-benzylaminopurine (BA) and indolebutyric acid (IBA). Shoot-tip explants was induced to produce multiple shoot initials in the presense or absence of apical domes, but the survival rates were higher when apical domes were retained. Cultivars varied widely in their multiplication rates in response to cytokinins, BA being consistently more effective than kinetin (Kn). Although Kn was less effective in this regard, it stimulated vigorous root growth.

Muhammad *et al.* (2004) cultured the shoot tips of banana cv. Basrai on Murashige and Skoog basal medium supplemented with 5.0 mg/l BAP and he recorded on the average, 124 plants produced from each shoot tip after five sub culturing.

Jing-Yan *et al*. (2011) added different hormonal combinations (0-6.0 mg/l 6-BA and 0.1-0.2 mg/l NAA) in MS medium to induce the adventitious buds in banana cv. Longxuan and to allow its multiplication. The best induction and multiplication medium for adventitious buds was found at MS + 3.0 mg/l 6-BA and MS + 4.0 mg/l 6-BA + 0.2 mg/l NAA, respectively.

Sipen and Davey (2012) studied the different concentrations of BAP and IAA for their effect on shoot multiplication and plant regeneration of the

Malaysian banana cultivars Pisang Mas, Pisang Nangka, Pisang Berangan and Pisang Awak. Maximum shoot was produced on medium supplemented with BAP at 5 mg/l (Pisang Nangka), 6 mg/l (Pisang Mas and Pisang Berangan) and 7 mg/l (Pisang Awak) with 0.2 mg/l IAA.

Rahman *et al.* (2013) investigated the best plant growth regulators for shoot proliferation and multiplication for cultivar Agnishwar. Among different types and concentration of cytokinins *viz*., BAP, kinetin (KIN), N6 - (2-isopentyl) adenine (2iP) tested for multiplication of shoot; maximum multiplication (95%) was obtained in MS medium containing 4.0 mg/l BAP. The highest average number of shoots for each explant (5.9) was found in MS medium fortified with 4.0 mg/l BAP while maximum elongation of shoot (4.9 cm) was observed in MS medium containing 5.0 mg/l BAP.

Ramachandran and Amutha (2013) carried out research work on Cavendish Dwarf variety of banana. Murashige and Skoog's basal medium supplemented with 4 mg/l BAP and 0.2 mg/l NAA was found to be most suitable combination for shooting. Further, for multiplication combination of BAP 5 mg/l + NAA 0.3 mg/l was found best while Ahmed *et al.* (2014a) found MS medium supplemented with BAP 4.00 mg/l + IAA 2 mg/l best for explant establishment and shoot multiplication of banana cv. Grand Naine.

Iqbal *et al.* (2013) develop a simple, comprehensive and efficiently repetitive protocol for micropropagation of banana (*Musa sapientum* L.) using shoot meristem. After growing *in vitro* cultures on different hormonal combinations, Murashige and Skoog's medium supplemented with BAP and IAA (5.0 + 1.0 mg/l, respectively) and 10% CW were found to be most efficient and productive combination for shoot proliferation.

Shiv Shankar *et al*. (2014) did mass propagation of banana (*Musa* spp.) cv. Grand Naine through direct organogenesis by using PGRs Benzyl Adenine

Purine and Kinetin. Benzyl Adenine Purine (BAP) in five different concentrations (control, 2.0, 4.0, 6.0, 8.0 and 10.0 mg/l) were used for shoot proliferation and differentiation and shoot multiplication rate. The study revealed that medium supplemented with BAP 4.0 mg/l produced greater number of shoots (55) and longer shoot (3.0±0.012 cm) when compared with other treatments.

Jamir and Maiti (2014) studied the effect of various levels of cytokinin and auxin for *in vitro* regeneration of banana cultivars Grand Naine and Jahaji. They tested various concentration of BAP (0-6.5 mg/l). 4.5 mg/l BAP was found as the best concentration in induction of highest no. of buds (an average of 7.05 and 7.2) with highest mean length of 0.65 cm and 0.7 cm of shoots. But, the shoot elongation was maximum at lower concentration of BAP (1.5 mg/l).

Saraswathi *et al*. (2014) conducted to devise an efficient method of micropropagation for a high yielding but recalcitrant banana cv. Udhayam (Pisang Awak, ABB) using shoot tip explants. Virus-indexed shoot tips were established in medium comprising full-strength MS basal salts and vitamins, supplemented with 50 mg/l ascorbic acid, 100 mg/l myo-inositol and 4.0 mg/l benzylamino purine (BAP). Among the various media tested for shoot proliferation, MS medium with BAP (3.0 mg/l) and 5% coconut water (CW) was found optimum as it produced the maximum number of 6.3 multiple shoots in a minimum period of 7.5 days.

Ahmed *et al.* (2014a) successfully conducted micropropagation method for banana Cv. Grand Naine, Suckers were surface sterilized with HgCl2 (0.1%) for 6 min which gave minimum contamination with maximum culture establishment. Of various treatment combinations, Murashige and Skoog (MS) medium + BAP 4.00 mg/l with IAA 2.00 mg/l resulted in maximum establishment of cultures in lesser time. MS medium + BAP 4.00 mg/l + IAA 2.00 mg/l gave maximum multiple shoots. Maximum rooting was obtained on

MS medium (half strength) supplemented with IBA 1.00 mg/l and activated charcoal 200 mg/l.

Mahdi *et al*. (2014) studied on two most favored banana (*Musa sapientum*) cultivars in Bangladesh *viz*., Anupam and Chinichampa. The experimental design was conducted to examine the effect of phytohormones *in vitro* on these cultivars. For *in vitro* study, field grown suckers were collected to isolate the meristemic part of the pseudostems and cultured in MS medium containing plant growth hormones after surface sterilization by 0.1% $HgCl_2$ containing BAP, kinetin (Kn), IAA and IBA for six weeks. Buds were sprouted when cultured in MS + BAP [4.5 mg/l] for Chinichampa, and when MS was supplemented with BAP [2.0mg/l] then 75% response was obtained with Anupam. Synergistic effect of cytokinin and auxin was found effective in improving shoot proliferation rate for both the cultivars. In Chinichampa, BAP [5.0 mg/l] + IAA [2.5 mg/l] showed 85% frequency with an average of 1.9±0.09 cm and in Anupam shoot length attained up to 2.9±0.11 cm in MS + BAP [5.0 mg/l] + IAA [1.5 mg/l]. Combination of BAP and Kn was found effective only in Anupam. Tallest shoot (3.5±0.09) was observed in MS + Kn [2.0 mg/l] + IAA [0.25 mg/l]. The regenerated micro-shoots were then cultured to induce roots in MS + IBA [1.0 mg/l].

Jane *et al*. (2015) studied the effects of cytokinins benzyl amino purine (BAP), 2-isopentenyl adenine (2iP) and kinetin each evaluated at 5, 10, 20, 24 and 40 µM and thidiazuron (TDZ) at 0.1, 0.5, 1 and 1.5 µM, on microshoot induction were investigated. The FHIA 17 explants cultured on MS media supplemented with 40 µM kinetin produced the highest (3.00±0.35) mean number of microshoots. On the other hand, INJAGI explants cultured on MS media supplemented with 0.1 µM TDZ yielded the highest mean number (1.84±0.24) of microshoots and the highest mean length (0.99±0.13).

Kale (2015) conduct an experiment on four cultivars of banana *viz*., Basrai, Grand naine, Ardhapuri and Srimanti for development of micropropagation protocol through shoot tip cultures. The response of Grand Naine cultivar was maximum. Whole plant regeneration protocol was successfully developed for all four cultivars with a success rate of 92-97%.

Khatun *et al*. (2017) conducted an experiment to study the effect of benzylaminopurine (BAP) (1.0, 2.0, 3.0, 4.0 and 5.0 mg/l) and indole-3-butyric acid (IBA) (0.5, 1.0, 1.5, 2.0 and 2.5 mg/l) for *in vitro* regeneration of Sabri variety of Banana using shoot tip explants. Highest response of explants (84%) and maximum number of shoots per explant (3.4) were observed with 5.0 mg/l BAP in Sabri. In contrast, due to combined effect, 5.0 mg/l BAP+2.5 mg/l IBA showed best response (90%). The highest shoot number per explant (3.4) was found with 5.0 mg/l BAP+2.0 mg/l IBA. The maximum number of roots (3.4 and 5.2) was observed in 1.5 mg/l and 3.0 mg/l BAP+1.0 mg/l of IBA.

Alango *et al.* (2018) cultured two banana cultivars on Murashige and Skoog (MS) medium supplemented with different concentrations of BAP and Kinetin sole and in combination for shoot initiation and multiplication. The highest multiple shoot (6.0 and 4.5/explants), in Poyo and Giant Cavendish were observed on the MS medium fortified with 2.5mg/ l BAP+2.5mg/l kin and 2.0mg/l BAP +2.0mg/l Kin respectively. For root induction 1.5mg/l IBA and 1.5mg/l IAA each tested separately on MS medium. IBA showed best performance with 5.12 and 4.69 root/ plantlet after four weeks of inoculation in Poyo and Giant Cavendish respectively. After 12 weeks in vitro plantlets were transferred to green house for acclimatization where 82% and 88% survival rate was recorded in Poyo and Giant Cavendish respectively.

Laxmi and Sahoo (2018) reported maximum callus induction of banana cv. Patakapura was on MS medium supplemented with BAP (8.0 mg/l) in combination with NAA (2.0 mg/l). Callus were multiply strongly on MS

medium supplemented with BAP (6.5 mg/l) in combination with NAA (1.6 mg/l). Additionally, maximum shoot proliferation and elongation were observed with BAP (5.5 mg/l) and NAA (1.2 mg/l) and better rooting response was observed in NAA (4.0 mg/l) with activated charcoal.

Hoque *et al*. (2018) reported minimum number of days required for shoot initiation (9.07) with highest shoot initiation percentage (91.14) and the longest shoot (2.23 cm) in MS medium supplemented with 5.0 mg/l BAP. On the other hand, highest shoot multiplication percentage (80.99) with maximum number of shoots per explant (4.47), the highest length of shoots (4.17 cm) and maximum number of leaves (4.04) was observed in MS medium supplemented with 4.0 mg/l BAP + 2.0 mg/l KIN + 2.0 mg/l NAA.

Rajoriya *et al*. (2018) studied on the effect of plant growth regulators on *in vitro* micro propagation of Indian red banana. They reported that 3 mg/l BAP + 0.2 mg/l NAA showed highest shooting. While the highest root length and no. of roots was observed with the 1 mg/l IBA.

Sivakumar and Visalakshi (2021) developed an efficient protocol for micropropgation of banana cv. Poovan by using shoot tip as explant. The explants were cultured on Murashige and Skoog (MS) medium containing different concentrations of benzyl amino purine (BAP) and thidiazuron (TDZ) for the development of shoots. The highest average number of shoots (7.1) for each explant was found in MS medium containing 1.0 mg l^{-1} TDZ, while, the maximum of five shoots were produced per explants in MS medium containing BAP (3 mg l^{-1}).

2.2 Studies on *in vitro* rooting

Karim *et al*. (2009) investigated the effect of different concentrations of BAP, NAA, IAA and IBA on regeneration, shoot proliferation and root formation in Banana (*Musa* spp). Meristems were collected from about four months old banana suckers were used on explants. In experiment 1, BAP (0.0,

2.5, 5.0, 7.5 and 10.0 mg LG) and NAA (0.0, 0.5, 1.0, 1.5 and 2.0 mg LG) were 1 1 used as treatments. It was observed that the highest number of root was produced by 0.5 mg LG IAA + 1 0.5 mg LG IBA (3.50, 4.50 and 6.50 per explant respectively), which was statistically significant than other 1 treatment. The highest length was observed at 10, 20 and 30 DAI in the treatment concentrations 0.5 mg LG 1 IAA and IBA (2.93, 4.63 and 5.88 cm respectively) which was statistically significant. However, the meristem having no or lowest concentration of growth regulators showed the lowest root development.

Govindaraju *et al*. (2012) found that the shoot proliferation was found best (80%) in the MS medium containing Benzylaminopurine (BAP) 2.0 mg/l. Maximum percent of adventitious root formation was observed in half strength MS medium supplemented with Indole butyric acid (IBA)1.5mg/l and Napthalene acetic acid (NAA) 1.0mg/l.

Sipen and Davey (2012) found that half strength MS medium fortified with 1 mg/l NAA was suitable for root regeneration from scalps in Malaysian banana cultivars Pisang Mas, Pisang Nangka, Pisang Berangan and Pisang Awak. They found maximum of 7 roots from cv. Pisang Nangka and Pisang Berangan and mean root length was also maximal (4.5 cm) in the latter two cultivars.

Bhosale *et al*. (2013) investigated the effect of different concentrations of growth regulators on rooting in different species of Banana such as Ardhapuri, Basrai, Shrimanti. The shoots which are developed on MS (Murashige and Skoog) medium was inoculated on same medium supplemented with combinations of growth regulators BAP (1, 3, 5, 7, 9mg/l) with IAA and IBA (1, 2, 3mg/l). With regard to different treatments the best rooting response was observed in BAP 1 mg/l +IBA 3mg/l.

Rahman *et al*. (2013) investigated the best plant growth regulator for induction of roots in banana cv. Agnishwar. Among different types and

concentration of auxin *viz*., Indole-3-Butyric Acid (IBA) and α-naphthalene acetic acid (NAA), IBA at 1.0 mg/l was found most suitable for rooting of shoot (96% rooting).

Anbazhagan *et al*. (2014) conducted an experiment for standardization of *in vitro* culture technique for the mass propagation of *Musa* sp. Shoot tips were used as explants and they were cultured on Murashige and Skoog (MS) medium supplemented with different concentrations of BAP, Kin and IAA both in individual and in combined form for shoot inductions and the best results were obtained from MS medium supplemented with BAP+IAA at the concentration of 3.0 mg/l and 0.5 mg/l respectively. Best root formation of in vitro developed shoots could be achieved on half strength MS medium supplemented with IBA at concentration 1.0 mg/l.

Ahmed *et al*. (2014a) obtained rooting on MS (half strength) medium fortified with IBA 1.00 mg/l and activated charcoal 200 mg/l.

Saraswathi *et al*. (2014) reported rooting in the MS medium fortified with indole butyric acid (IBA) 0.5 mg/l + naphthalene acetic acid (NAA) 1.0 mg/l + activated charcoal (AC) 250 mg l-1 for banana cv. Udhayam (Pisang Awak, ABB) using shoot tip explants.

Jamir and Maiti (2014) studied the effect of IBA and NAA on rooting of banana cultivars Grand Naine and Jahaji. For rooting, NAA and IBA were used individually at the concentration of 0, 0.1, 0.2 and 1 mg/l. Cent percent rooting and also highest no. of functional roots (6.33 and 5.20) with moderate root length (2-4 cm) were achieved on medium with 1 mg/l NAA.

Jane *et al*. (2015) studied the effects of auxins *viz*., napthaleneacetic acid (NAA), indole-3-acetic acid (IAA), 2, 4-dichlorophenoxyacetic acid (2, 4-D) and indole-3-butyric acid (IBA each evaluated at 5, 10, 20 and 40 µM) on rooting. Rooting was achieved in all media supplemented with the auxins evaluated except on 2, 4-D.

Miilion *et al*. (2015) undertook a study to determine the effect of different concentrations of growth regulators of IAA (indole-3-acetic acid) (0.0, 0.5 and 1.0 mg/l) and IBA (indole-3- butyric acid) (0.0, 0.5, 1.0 and 1.5 mg/l) and their interaction on rooting. Half strength MS medium was used for all treatments. The highest numbers of roots were produced by treatment 0.5 mg/l IAA which was 6.2 and 7.8 at 15 and 30 DAI, respectively. 1.5 mg/l IBA was produced 5.1 and 7.1 roots at 15 and 30 DAI respectively. In 0.5 mg/l IAA + 1.5 mg/l IBA combination was given 7.0 and 8.0 numbers of roots at 15 and 30 DAI respectively. In length of roots, it was observed that 0.5 mg/l IAA was produced longest root size, 5.7 and 6.7 cm at 15 and 30 DAI respectively. The same concentration of IBA produced 4.5 and 5.9 cm root length at 15 and 30 DAI respectively. In interaction, 0.5 mg/l IAA +0.5 mg/l IBA were produced 6.33 and 7.33 cm length in 15 and 30 DAI respectively.

Shashikumar *et al*. (2015) found that MS medium supplemented with 1.0 mg/l IBA induced 5.33 ± 1.21 number of roots with a mean root length of 7.50 ± 1.87 cm in banana cv. Karibale Monthan.

Dahari *et al*. (2017), studied root induction ½MS media under various concentrations of IBA indicated that the more numbers of roots were recorded (1.25 and 2.61) under the concentration of 1.50 mg/l IBA at 20 and 40 days, while 3.67 numbers of roots was achieved at 60 days with concentration of 1.0 mg/l IBA.

Hoque *et al*. (2018) reported best result on days required for root initiation (9.00), the highest root initiation percentage (85.05), maximum number of roots per plantlet (5.83) and the highest length of roots (4.17 cm) was obtained in MS medium supplemented with1.5 mg/l IBA + 0.5 mg/l IAA.

Ajie *et al*. (2019) studied on increasing the production of Raja Bulu banana by *in vitro* culture with addition of coconut water and NAA. The experimental design was Completely Randomized Design (CRD) two factors:

coconut water (K) with concentration K1 = 50 ml l^{-1}, K2 = 100 ml l^{-1}, K3 = 150 ml l^{-1}, and NAA (A) with concentration A0 = 0 ppm; A1 = 0.5 ppm; A2 = 1 ppm; A3 = 1.5 ppm. The result showed that combination of coconut water at 50 ml l^{-1} and NAA 0,5 ppm resulted in highest number of shoots (5.6 shoots). Medium supplemented with coconut water 100 ml l^{-1} and NAA 0 ppm resulted in highest number of leaves (8 leaves). In the medium treated with NAA 1.5 ppm, shoots appeared after six days of planting and yielded the highest number of roots (11.55 roots). Coconut water increased the shoot height at concentrations of 50 ml l^{-1}, accelerated root formation at concentrations of 100 ml l^{-1}, and increased number of roots at concentrations of 50 ml l^{-1}.

Doaa *et al*. (2021) investigated the influence of increasing copper levels in an MS medium on shoot multiplication; rooting and the acclimatization of *in vitro* cultured banana. Four different concentrations of copper sulphate (0.025 as a control, and 30, 60, and 120 mg/l) were examined. At 0.025 mg/l of copper sulphate optimal shoot number and shoot length (10 shoots and 6 cm, respectively) were observed without significant differences at 30 mg/l. The root length of banana plantlets was significantly enhanced at 30 mg/l of copper sulphate but without significant differences to the control, regarding the number of roots (9.92 cm and 3.80 roots, respectively). *In vitro* plants were acclimatized successfully at 30 mg/l of copper sulphate with 100% survival.

Sivakumar and Visalakshi (2021) developed an efficient protocol for micropropgation of banana cv. Poovan by using shoot tip as explant. The explants were cultured on Murashige and Skoog (MS) medium containing different concentrations of inodole butyric acid (IBA) for root induction. More number of roots was produced in the medium having IBA (1.0 mg l^{-1}).

2.3 Studies on male inflorescence

Resmi and Nair (2007) reported *in vitro* micropropagation of diploid cultivar (Sannachenkadali, AA) and triploid cultivar (Red banana, AAA) using

inflorescence apices as explants. The diploid cultivar induced a maximum number of multiple shoots in 8.9 μM 6-benzyl adenine (BA) whereas the triploid cultivar exhibited maximum multiplication in 22.2 μM 6-benzyl adenine. MS medium supplemented with 11.4 μM indole acetic acid and 17.8 μM BA was also suitable for shoot proliferation in triploid cultivar but not in the diploid cultivar. The regenerated shoots were rooted in Murashige and Skoog basal medium within 10–15 days. The rooted plantlets were transferred to vermiculite and maintained at a temperature of 25 ± 2°C for 10 days and then at room temperature (30–32°C) for 2 weeks before transferring to potted soil compost mixture. The plantlets showed 100% survival.

Mahadev *et. al*. (2011) studied on different explant sources for banana micro- propagation such as suckers, shoot tips, and floral buds on hill banana ecotypes (AAB) 'Virupakshi' and 'Sirumalai. Immature male floral buds were collected from healthy plants from hill banana growing areas. Exposure of explants to ethyl alcohol (70%, v/v) for 30 s, then mercuric chloride (0.1%, w/v) for 30 s, followed by three independent rinses of 5 min each in autoclaved, double-distilled water satisfactorily reduced the contamination. Male floral bud explants were cultured on Murashige and Skoog (MS) basal medium supplemented with different combinations of 6-benzylaminopurine (BAP), coconut water, naphthaleneacetic acid, gibberellic acid, and additional supplements. MS medium supplemented with 5 mg l^{-1} BAP and coconut water (15%) was the most efficient media for shoot initiation and multiple shoot formation (15 shoots from a single part of a floral bud). The best response for shoot elongation was obtained using the combination of basal MS, 5 mg l^{-1} BAP, 1 mg l^{-1} naphthaleneacetic acid and 1.5 mg l^{-1} gibberellic acid. Regenerated shoots were rooted in basal MS medium within 15–20 d. The rooted plantlets were transferred to a soil mixture and maintained at a temperature of 25 ± 2°C for 10 d and then at room temperature (30–32°C) for 2 wk, before transferring to a greenhouse. The regenerated plantlets showed

100% survival.

Hossain *et al*. (2013) investigated the effect of three different carbon sources; sucrose, glucose and sorbitol alone and their combinations on *in vitro* callus induction and regeneration in banana cv. *Sabri*. Male flowers were used as experimental material in this investigation. The male flowers were cut into small pieces and cultured on MS medium supplemented with 2 mg/l 2, 4-dichloro phenoxy acetic acid (2, 4-D) + 1 mg/l a-naphthalene acetic acid (NAA) + 1 mg/l Indole-3-acetic acid (IAA) + 1 mg/l biotin+1 mg/l glutamine containing different percentages of sugars like sucrose, glucose and sorbitol singly and in their combinations. Glucose showed the highest performance rate for callus induction and 3 % concentration proved the optimal dose. Sucrose and sorbitol behave different in embryo formation and they produced the highest and lowest number of embryos, respectively in regeneration medium. In respect of overall performance the highest percentages shoot and root formation was obtained in the media containing 3% and 2% sucrose, respectively.

Hrahsel *et al*. (2014) developed an efficient *in vitro* propagation method for *Musa acuminata* (AAA) cv. Vaibalhla, an economically important banana cultivar of Mizoram, India using immature male flowers were used as explants. Out of different PGR combinations, MS medium supplemented with 2 mg l^{-1} 6-benzylaminopurine (BAP) + 0.5 mg l^{-1} α-naphthalene acetic acid (NAA) was optimal for production of white bud-like structures (WBLS). On this medium, explants produced the highest number of buds per explant (4.30). The highest percentage (77.77) and number (3.51) of shoot formation from each explants was observed in MS medium supplemented with 2 mg l^{-1} kinetin + 0.5 mg l^{-1} NAA. While MS medium supplemented with a combination of 2 mg l^{-1} BAP + 0.5 mg l^{-1} NAA showed the maximum shoot length (14.44 cm). Rooting efficiency of the shoots was highest in the MS basal medium without any PGRs. The plantlets were hardened successfully in the greenhouse

with 96 % survival rate.

Juhua *et al*. (2016) studied an efficient regeneration system applicable to five *Musa* Cultivars on MS medium supplemented with 8.9 µ mol l^{-1} BA. In 240–270 days, one immature male flower could regenerate between 380 and 456, 310–372, 200–240, 130–156, and 100–130 well-developed shoots for Gongjiao, Red banana, Rose banana, Baxi, and Xinglongnaijiao, respectively.

Nair *et al.* (2018) developed to multiply *Musa paradisiaca* cv. Poovan using male immature flower bud. Size of explants has been found an influencing factor for culture initiation. Immature male flower bud segments of 3 cm size were ideal for better survival and subsequent shoot regeneration. Direct shoot regeneration was achieved from male immature flower buds on Murashige and Skoog (MS) medium supplemented with varying concentrations of plant growth regulators. Initially, actively dividing meristematic region developed at the basal region of flower buds near the bract axil, which later grew into green shoot buds in most of the PGR treatments. Single use of benzyl adenine were found beneficial than kinetin or addition of indole-3-acetic acid. Maximum production of 31.0 ± 0.65 shoots was achieved on MS + 3% sucrose + 6 mg/l benzyl adenine in 15 weeks. Isolated healthy shoots were rooted in half-strength MS medium with 150 mg/l activated charcoal + 30 g/l sucrose + 1 mg/l indole-3-butyric acid within 15 days and they established successfully in greenhouse conditions with 85 % survival.

Nandariyah *et al*. (2021) conducted a study to regenerate banana flowers *in vitro* with different sucrose and BA (Benzyladenine) concentrations. The study used a Completely Randomized Design (CRD), two factorials sucrose concentration with 4 levels (20 g/l, 25 g/l, 30 g/l, and 35 g/l) and BA concentration with 4 levels (2 ppm, 4 ppm, 6 ppm and 8 ppm). The results showed that the combination of BA and sucrose concentration had not directly induced organogenesis in banana flower explants. Growth and development of banana flower explants maximally form Cauliflower-Like Bodies.

2.4 Study on effect of different carbon sources

Buah *et al*. (2000) and Kadota and Niimi (2004) investigated the influence of sorbitol, sucrose, fructose, glucose, maltose, lactose and mannitol carbon sources at various concentrations on shoot proliferation, hyperhydricity and rooting of pear. Sorbitol at 60 mM was the most effective carbon source for shoot proliferation. Shoots rooted with 60 mM glucose, sucrose and sorbitol media. However, media with sucrose resulted in the highest rooting frequency, root no. and root length. Shoots failed to root when fructose, lactose maltose and mannitol were used**.**

Madhulatha *et al*. (2006) studied the effects of carbon sources (sucrose, glucose, fructose and mannitol) and auxins (IBA) and (NAA) on *in vitro* propagation of banana (*Musa* spp. AAA). Of all carbon sources tested, sucrose induced highest frequency of shoot proliferation. Optimal shoot proliferation rates were achieved on the MS medium supplemented with sucrose and glucose combination (1:1) at the concentration of 30 g dm^{-3}

Hossain *et al*. (2009) reported on the comparative performance of different carbon sources (glucose, sucrose and sorbitol) in cell growth and regeneration efficiency of banana (*Musa* spp.) cultivars. Glucose showed the highest performance in callus induction and cell growth and 3% glucose proved as the optimal dose in media formulation for callus induction and cell growth. Sucrose and sorbitol behaves differently in embryo formation and they produced the highest and lowest number of embryos respectively in regeneration medium. In respect of overall performance the highest percentages of shoot and root formation was obtained in the media containing 3% sucrose. Glucose proved to be the best carbon source in callus induction and cell growth media.

Vora and Jasrai (2011) studied the effect of various carbon sources on *in vitro* shoot multiplication of banana, Grand naine. They observed highest rate

of shoot-multiplication can be obtained in the medium containing fructose (3%) instead of sucrose (3%).

Yaseen *et al*. (2013) reviewed the role of carbon sources for *in vitro* plant growth and development. *In vitro* plant cells, tissues and organ cultures are not fully autotrophic establishing a need for carbohydrates in culture media to maintain the osmotic potential, as well as to serve as energy and carbon sources for developmental processes including shoot proliferation, root induction as well as emission, embryogenesis and organogenesis, which are highly energy demanding developmental processes in plant biology. A variety of carbon sources (both reducing and non-reducing) are used in culture media depending upon genotypes and specific stages of growth. However, sucrose is most widely used as a major transport-sugar in the phloem sap of many plants. In micropropagation systems, morphogenetic potential of plant tissues can greatly be manipulated by varying type and concentration of carbon sources

Reddy and Harika (2012) investigate the effect of sucrose at different concentrations as a carbon source in modified MS 1962 medium, on the growth and propagation of Grand naine (*Musa* spp.) plantlets. Six types of media [modified Murashige and Skoog (1962)] were prepared, which were supplemented with various concentrations of sucrose to evaluate the effect of sucrose and its effectiveness in propagation of the Grand naine plantlets. Plants that were cultured on 30 gl^{-1} sucrose have higher fresh weight and shoot length, and high competence in propagation compared to those that were culture on six types of media. In this amend 30 gl^{-1} of sucrose as the optimum concentration for the essence propagation, growth and multiplication of Grand naine when compared to other six types of media.

Ilczuk *et al*. (2013) on a study in efficiency of shoot proliferation and *in vitro* rooting of common Ninebark reported that efficient plant micropropagation depends upon a number of factors one of which is the type and concentration of exogenously supplied carbon sources in the medium.

Fructose, glucose, maltose and sucrose were tested at concentration ranging from 0-50 g dm^{-3}. Sucrose did not stimulate shoot proliferation and glucose was completely ineffective in rooting induction. The highest number of shoots was produced on the fructose-containing medium. The concentration of 30 g dm^{-3} appeared to be optimal; the rate of proliferation at 30 and 40 g dm^{-3} were in fact similar, but the former produced a more favorable shoot length. The number of adventitious roots produced per shoot increased with increasing fructose concentration up to 30 g dm^{-3}.

Ahmed *et al.* (2014b) investigated the effect of different carbohydrate source, pH and supporting media on *in vitro* rooting of banana plantlets. In the absence of sucrose culture could not survive after 3 weeks of incubation. Media containing sucrose 30 g/l gave the best result. Sucrose in the medium remarkably influenced the rooting of plantlets.

Brearley *et al.* (2014) optimized micropropagation protocols to minimize hyperhydricity were for medicinal *Scutellaria barbata* and *Scutellaria racemosa*. They studied on six cytokinins and eight different carbon sources. In *S. barbata* nodal explants cultured on shoot induction medium supplemented with fructose and glucose produced 10 and 9 adventitious shoots respectively; and after 21 day incubation adventitious shoot count reached 19 in glucose supplemented medium. Similarly in *S. racemosa* explants in the same experiment produced 5 shoots in maltose and 4 shoots in sorbitol supplemented medium after 14 day incubation; whereas after 21 day incubation, sucrose and maltose produced 5 shoots; fructose, glucose, and sorbitol produced 4 shoots.

Mahadev *et al*. (2014) conducted *in vitro* experiments on the effect of various carbon sources such as sucrose, glucose, fructose and maltose on *in vitro* shoot regeneration of *Solanum viarum* using axillary bud explants. The frequency, growth and multiplication rate were highly influenced by the type and concentration of carbon source used. Among the different concentrations

(1% - 6%) of carbohydrates studied, the maximum number of shoots (22.6 ± 0.50) and shoot length (5.92 ± 0.13 cm) was obtained on MS medium supplemented with 4% (w/v) fructose. The least number of shoots (1.5 ± 0.32) was obtained on 6% maltose and the least shoot length (1.2 ± 0.23) was observed on 6% glucose. Among the four types of carbon sources that were employed in the study, fructose at 4% proved to be better choice for multiple shoot regeneration followed by sucrose, glucose and maltose from axillary bud explants of *S. viarum*.

Morfeine (2014) reported the effect of sugar concentrations (sucrose and glucose) on micropropagation of *Musa in vitro* the concentrations of sucrose were used is 0 (control), 15, 30, 45, 60 and 75 g/l sucrose. And the concentrations of glucose were used is 30g/l sucrose (control), 15, 30, 45, 60 and 75 g/l glucose. Ten (10) replicates were used for each treatment. Results indicated that the 30 g/l and 45 g/l sucrose and 45 g/l and 60 g/l glucose show significant increase in micro-propagation of *Musa*.

Waman *et al*. (2014) studied the *in vitro* multiplication, rooting, and acclimatization of banana as influenced by carbon source-concentration interactions in 'Silk' banana (AAB). They reported that Fructose (2%) followed by sucrose (3%) were found to be most the congenial carbon sources for obtaining the highest shoot multiplication rates; Growth parameters were also found to be superior in the fructose-containing media; however, it performed poorly in terms of root induction. Whereas, medium containing sucrose (3%) supported 100% root induction of *in vitro*-derived shoots.

Manawadu and Dahanayake (2015) conducted *in vitro* shoot regeneration of Rabu (*Raphanus sativus* L.) on various carbon sources such as sucrose, glucose, maltose and dextrose. The frequency, growth and multiplication rate were highly influenced by the type and concentration of carbon source used. The maximum number of shoots (6 shoots/explant) on MS medium supplemented with 3% dextrose and maximum shoot length (4cm) was

obtained by 4% dextrose. Regeneration frequency was 100% in 4% dextrose. The least number of shoots were observed by each and every concentration of glucose except 4%.

Qamar *et al*. (2015) reported the effect of various concentrations of sucrose on *in vitro* rooting of regenerated banana micro shoots. Three selected *Musa* varieties GCTCV-215 (AAA), Yangambi (AAA) and FHIA-23 (AAAA) were cultured in half strength MS media supplemented with root promoting growth regulator 1.0 mg/l-1 IBA and different concentrations of sucrose (0, 20, 40, 60 and 80 g/l^{-1}). Significant differences ($p \leq 0.05$) were noticed in all *Musa* clones. Results indicated that as compared to control out of different concentrations of sucrose all varieties, showed the maximum number of roots as well as highest root length in MS medium augmented with 40 % and 20 % g/l sucrose respectively.

Bohra *et al*. (2016) reported the effect of kind and concentration of carbon source on concurrent *ex vitro* rooting and hardening (CEVRH) in Ney Poovan banana. They found out that kind and concentration of carbon source present in the nutrient pool at the time of multiplication had a significant effect on the *ex vitro* performance of the plantlets. Better *ex vitro* rooting and hardening performance was exhibited by the shoots obtained from glucose (2%) supplemented medium.

Nabil *et al*. (2016) examined the effect of plant growth regulators, gelling agents and sucrose on micropropagation and hyperhydricity of *Gypsophila paniculata* L. Shoot tips were tested at different levels and combinations of 6-benzyl adenine (BA) and α-naphthaleneacetic acid (NAA) for shoot induction. Shoot proliferation and hyperhydricity were evaluated under different levels of sucrose, agar and gelrite. Highest efficiency of shoot formation was obtained with Murashige and Skoog's (MS) medium containing 0.5 mg/l each of BA and NAA. Here, 98.7 % explants produced 7.5 shoots per shoot tip with 4 cm. length per shoot. The lowest degree of hyperhydricity

(13.4 and 8%) was recorded when 8 and 10 g/l agar, respectively, were used. Higher concentrations of sucrose (40-60 g/l) recorded inhibitory effect on shoot formation, shoot number, shoot length and reduced hyperhydricity rate. For *in vitro* rooting, half strength MS media supplemented with different combinations of NAA and 3-indolebutyric acid (IBA) were tested, out of which 1 NAA + 3 IBA mg/l and 3 mg/l IBA were found to be favorable with 95 % rooting.

Trettel *et al*. (2018) conducted a study on *in vitro* growth of Genovese basil in response to different concentrations of salts in the Murashige and Skoog medium (MS) and interaction of sucrose and activated carbon. Doubling the usual dose of sucrose (60 g/l) damaged the growth of the seedlings. Damage caused by osmotic and oxidative potentials, and by toxic compounds may be related to the observed results.

Jeong and Sivanesan (2018) investigated the Influence of sucrose concentration on the adventitious shoot regeneration in shoot cultures of *A. multiflora*. The highest number of shoot buds (34.80) per leaf was obtained in shoot induction medium supplemented with 2% sucrose. The maximum content of total carotenoids was achieved in shoots grown on MS medium with 3% sucrose. The highest content of total tocopherol was achieved in shoots cultured on MS medium with 3% sucrose under WFL, while the lowest content of total tocopherol was obtained in shoots cultured on MS medium containing 1% sucrose under red LED.

Isaac *et al*. (2018) investigated the effect of three different concentrations of sucrose namely 2, 3 and 4% on the *in vitro* regeneration of embryo explants of *T. africana* Decne. on the media of Murashige and Skoog (MS) and (B5) respectively without any growth regulator. 4% sucrose elicited the best response in all the growth parameters determined while control gave the least response.

Memon *et al*. (2019) studied three carbon sources such as (dextrose, sucrose and sorbitol) used to increase Murashige and Skoog medium at four applications control, 15, 30, 45 and 60 g/l respectively. Randomized Block Design (RBD) method with 3 replications was used for each treatment. Results indicated that sucrose give maximum result as compared to dextrose and sorbitol. Though, significant result was found in sucrose 30 g/l as compared to dextrose and sorbitol concentrations.

2.5 Studies on acclimatization of plantlets

Acclimatization is necessary in the case of *in vitro* produced plantlets because *in vitro* produced plant material is not adapted to natural environmental conditions. They are very poorly adopted to resist the low humidity, higher light levels and more variable temperature prevailing outside (Wainwright, 1988).

Rai *et al*. (2012) hardened rooted plantlets of banana variety Grand Naine (G9) in portrays containing different potting mixtures *viz*., soil, sand and cocopeat (1:1:1), soil sand and farmyard manure (1:1:1) and mixture of cocopeat and sand (2:1) of which, the mixture of Cocopeat and sand (2:1) showed maximum (96%) survival of plantlets.

Shiv Shankar *et al*. (2014) found the survival rate of the plantlets in coconut coir pith to be 84.44% during primary hardening. All the plantlets were subjected to the secondary hardening with garden soil, sand and red soil in the ratio of 1:1:1 in polybags and all the plantlets showed 100% survivability.

Jamir and Maiti (2014) recorded that *ex-vitro* survival of cultured banana var. Robusta plantlets was 98% when they were subjected to secondary hardening in media mixture of presterilized topsoil: FYM: sand: vermicompost: cocopeat in ratio of 1:1:1/2:1/2:1 (V/V) filled in black polybags under 75% shade net condition.

Parkhe *et al.* (2018) conducted a detailed study on different potting mixture on hardening of banana tissue culture plantlets cv. Grand Naine. Different potting mixture of garden soil (control), garden soil+cocopeat (3:1), garden soil+ farm yard manure (3:1), garden soil+vermicompost (3:1), garden soil+ cocopeat+ FYM+ vermicompost (2:1:1:1) and garden soil+ sand+ FYM+ cocopeat (2:1:1:1) were used for secondary hardening. The in-vitro rooted plantlets were hardened and acclimatized by using different treatments. Plantlets were transplanted from primary hardening after 45 days primary hardening gave maximum survival (100%) during transplanting in the field. These plants were hardened in polythene bags singly. The maximum survival during hardening (100%) was observed in shade net with maintained relative humidity and light intensity. Various potting mixtures were tried, the potting mixture containing garden soil and FYM (3:1) gave maximum height and survival of plantlets and shows outstanding performances in field condition.

Parkhe *et al*. (2019) evaluated that the Ex Agar plant can be successfully hardened to produce the healthy planting material in two stages hardening, (Primary and Secondary hardening) by the cost effective process. In that primary hardening media (cocopeat+ perlite) and secondary hardening mixtures containing different potting mixture of garden soil (control), garden soil+ cocopeat (3:1), garden soil+ farm yard manure (3:1), garden soil+ vermicompost (3:1), garden soil+ cocopeat+ FYM+ vermicompost (2:1:1:1) and garden soil+ sand+ FYM+ cocopeat(2:1:1:1) showed that cocopeat was best medium for primary hardening for plantlets survival (95 %), In secondary hardening plant height 30.86 cm, pseudostem girth 2.15 cm, number of leaves 7.15, leaf area 417.63 cm^2, root length 28.20 cm, root mass 29.17 gm and 100 % survival was recorded in treatment garden soil+ FYM (3:1) whereas garden soil + vermicompost (3:1) recorded best results for secondary hardening. Cocopeat+ perlite for primary hardening and combination of garden soil+ FYM (3:1) was best followed by garden soil + Vermicompost (3:1) medium for

primary and secondary hardening respectively *in vitro* propagated banana.

Tarek *et al*. (2022) conducted a study to avoid the environmental problem resulting from the chemical fertilizers; nanofertilizers of Se and Cu were separately applied during the acclimatization of banana. The biological form of nano-Cu (50 and 100 mg/l) and nano-Se (25, 50, 75, and 100 mg/l) were studied on acclimatized banana transplants under greenhouse conditions. Both applied nanofertilizers enhanced the growth of transplant by 10.9 and 12.6% for dry weight after nano-Se and nano-Cu application up to 100 mg/l, respectively. The survival rate was also increased by increasing applied doses of both nanofertilizers up to 100 mg/l, whereas the highest survival rate (95.3%) was recorded for nano-Cu. All studied photosynthetic pigments and its fluorescence were improved by applying nanofertilizers. Studied antioxidant enzymatic activities (CAT, PPO, and POX) were also increased. A pH decrease in the growing medium was noticed after applying nano-Cu, which may explain the high bioavailability of studied nutrients (N, P, K, Cu, Fe, Se, and Zn) by banana transplants.

2.6 Studies on alternative Low Cost Media (LCM)

Agrawal *et al.* (2010) worked to decrease the cost of *in vitro* conservation of banana cv. Karpura Chakkarakeli (AAB; Mysore subgroup) without any adverse effects on cultures, expensive components of medium such as sucrose and gelling agents, *i.e*., phytagel or agar (90% of the total cost of the medium), were replaced with inexpensive alternates such as market sugar and isabgol, respectively. In general, no significant effects of isabgol and market sugar were observed on shoot (1.0-1.3 shoots/shoot explant) and root (1.5-2.0 roots/shoot explant) regeneration. The total cost of medium used for *in vitro* conservation of banana was decreased by 59% by using isabgol as an alternate gelling agent to agar and phytagel.

Gitonga *et al*. (2010) evaluated a micropropagating protocol for local banana (*Musa* spp.) in Kenya as an alternative to reduce the unit cost of micropropagation. Matrices were satisfactory and comparable to the gelling agents. Glass beads were, however, the best matrix in shoot multiplication. Use of support matrices, locally available macronutrients, micronutrients, sugar, equipment and facility reduced the cost of consumable material for banana tissue culturing by about 94%. Putting into account energy, labour and capital investments, the cost dropped from approximately US $ 1.5 to 1.0 per plantlet. Contamination was not observed when the media and equipment were sterilised using a pressure cooker instead of an autoclave. Use of plastic syringes instead of glass cylinders and micropipettes, to measure volumes reduced the cost of the equipment by 96%. The risk of damage and loss due to breakage was eliminated compared to the use of glassware equipment. Shoots were rooted when they were transferred to Murashige and Skoog (MS) medium supplemented with 1 mg l^{-1} napthaleneacetic acid (NAA) or 1 mg l^{-1} Anatone.

Placide *et al*. (2012) investigated the effects of table sugar, laboratory grade sucrose and mannitol on growth of banana plantlets under *in vitro* conditions. Their study indicated a possibility of replacing the laboratory grade sucrose by the table sugar without significant loss in quality and growth so as to reduce the production cost of *in vitro* plantlets of banana.

Gabriel *et al*. (2013) formulate a culture media using low-cost materials as substitute to chemical components, such as Murashige and Skoog (MS) with the addition of 5ppm Benzyl Amino Purine (BAP) 2% sucrose and 4.5g Biolife agar. Substituting the chemical formulation led to comparable results in terms of the growth of plantlets as well as the quality and quantity of plantlets. The developed culture media was used for shoot proliferation and root induction of *in vitro* banana. The cost of one shoot/meriplant was P0.02 using the developed media, compared to the P0.20 using the chemical formulation. Likewise, the costs of rooting one plantlet were P0.64 and P0.26 for the existing and for the

developed media, respectively. Hence, the total cost of producing one plantlet (from shoot proliferation to root induction) was P0.28 via the developed media, while that of using the existing media was P0.84.

Dhanlakshmi and Stephan (2014) conducted a study to reduce the cost of banana tissue culture nutrients by using alternative nutrients sources. The conventional sources of Murashige and Skoog (MS) media were replaced by mixed nutrients containing both macro and micronutrients. The mixed nutrients were supplemented with 30 g/l of table sugar and 8 g/l agar. The conventional MS medium supplemented with 30 g/l sucrose and 8 g/l agar was used as control. Two banana varieties of Poovan and Monthan were regenerated on the two media. There was 61.4% reduction in the cost of the nutrients used in the media preparation. Significant differences were detected on the number of shoots produced by Poovan on the two media with shoots cultured on the low cost medium producing average of four shoots per plantlets.

Saraswathi *et al*. (2016) developed a Cost-effective tissue culture protocols for the commercial multiplication of three banana varieties, 'Rasthali' (AAB-Silk), 'Grand Naine' (AAA-Cavendish), and 'Udhayam' (ABB-Pisang Awak). Three different carbon sources [table sugar, rock candy, and small candy (EID Parry India Ltd, Chennai, India)] were tested, each at three different concentrations [3.0%, 4.0%, or 5.0% (w/v)] in preliminary trials, with 3.0% (w/v) tissue culture-grade sucrose (Hi-Media, Mumbai, India) as the control. Table sugar at 3% (w/v) resulted in early greening and produced the maximum number of shoots per explant (5.75), followed by 3% (w/v) rock candy (3.75), and 4% (w/v) small candy (1.75) in 'Udhayam'. A 95% reduction in cost was achieved by replacing laboratory-grade sucrose with commercially-available table sugar during media preparation.

Prabhuling and Sathyanarayana (2017) studied on some inexpensive and readily available sources of carbon such as laboratory grade sucrose, common

grade sugar, cube sugar, rock sugar, candy sugar, glucose, jaggery and sugarcane juice were evaluated for *in vitro* propagation of banana cv. 'Grande Naine'. Best response in terms of shoot multiplication and rooting were achieved with rock sugar and common grade sugar, respectively which could be compared well with that of analytical grade sucrose. The results showed the possibility of successful use of cheaper carbon sources for micropropagation of banana cv. 'Grande Naine'.

CHAPTER III

MATERIALS AND METHODS

MATERIALS AND METHODS

The present investigation entitled “Responses of various carbon sources for *in vitro* regeneration of banana cv. Chinichampa (AAB)” was carried out at the Tissue Culture Laboratory, Department of Horticulture, School of Agricultural Sciences, Nagaland University, Medziphema Campus during the year 2019-2022. The details of materials used and the experimental methods followed during the course of the investigation are presented in this chapter.

3.1 Materials

3.1.1 Genotype

Chinichampa (AAB genome) is one of the hardiest and tallest cultivar grown in North Eastern parts of India. Its fruits are small and have a thin peel. The pulp is creamy in colour and its taste is sub – acid. The fruit turn golden yellow when ripe and keep well. The bunch contains 150-250 fingers and weighs about 16 kg.

3.1.2 Explant source

Two types of explants of Chinichampa (AAB) were used for *in vitro* regeneration studies. They are-

- Suckers (2-3 months)
- Immature male floral buds (MFBs)

3.1.3 Carbon source

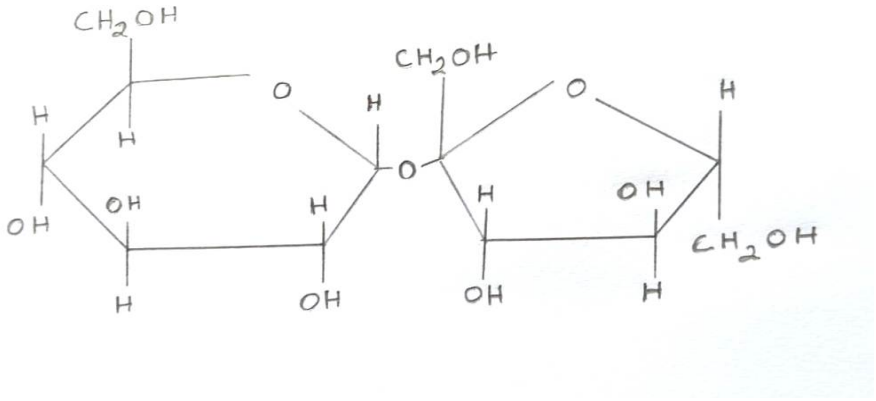
A carbohydrate is an indispensable ingredient of all culture media, as the photosynthetic ability of the cultured tissues is limited because of its light intensity and limited gas exchange (Kozai, 1991). It is also required as an osmotic agent (Thorpe, 1985). Although sucrose is the commonly used carbohydrate in the vast majority of work on in vitro shoot induction and development in woody species, it is not always the most effective carbon source for these purposes (Thomson and Thorpe, 1987). Prakash (1993)

concluded that sucrose accounts for 21.7 per cent of the media cost. Some reducing sugars such as glucose proved to be an efficient carbon source in tissue cultures of Malus and Prunus species (Borkowska and Szczerba, 1991. Therefore, the carbohydrate requirements must be defined and optimized for each micropropagation system.

Eight different sources of carbon were used for the present study. The properties of these carbon sources are given below.

3.1.3.1 Sucrose ($C_{12}H_{22}O_{11}$)

It is a non-reducing disaccharide, meaning it contains -D-Fructofuranosyl and L-D Glocopyranosyl and has a molecular weight of 342.30 g/mol (Glessener, 1984). It is hygroscopic, one gram dissolves in 0.5 ml of water. It is hydrolyzed to glucose and fructose by dilute acid and by the enzyme invertase. It contains 40 per cent carbon, 6.48 per cent hydrogen and 51.42 per cent oxygen and is obtained from sugarcane and sugarbeet. In the present study, the sucrose (Saccharose) LA grade (HiMedia Laboratories Pvt. Ltd. Mumbai, India) were used at 30 g/l of the medium in our experiments.

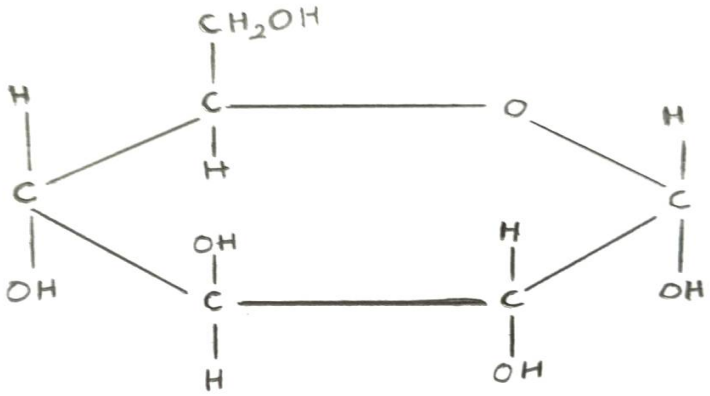


Sucrose Structure

3.1.3.2 Glucose $C_6H_{12}O_6$

It is a monosaccharide, mostly D-glucose. Its molecular weight is 180.16 and contains 40 per cent carbon, 6.72 per cent hydrogen and 53.29 per cent oxygen. Also known as blood sugar occurs naturally in the free states in fruits

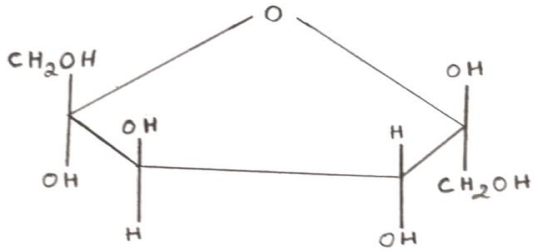
and other parts of plants. It is 0.74 times as sweet as sucrose. One gram dissolves in about one ml of water (Glessner, 1984). Glucose powder (dextrose) is white or cream coloured crystalline powder obtained by the complete hydrolysis of starch. In the present study, the glucose (D-(+)-Glucose anyhydrous) LA grade (HiMedia Laboratories Pvt. Ltd. Mumbai, India) was used as carbon source at 20, 30 and 40 g/l of the medium in our experiments.



Glucose Structure

3.1.3.3 Fructose $C_6H_{12}O_6$

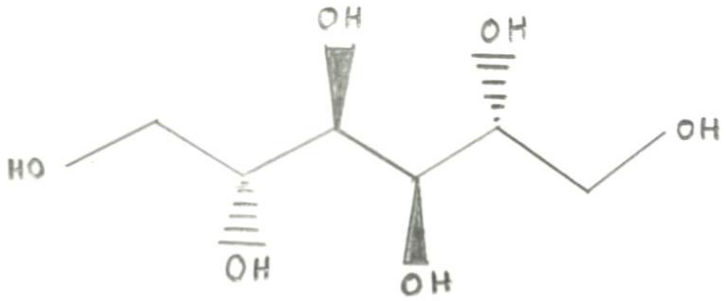
It is a monosaccharide, mostly D-fructose. Its molecular weight is 180.16, contains 40 per cent carbon, 6.72 per cent hydrogen and 53.29 per cent oxygen (Glessner, 1984). It is also known as fruit sugar, occur naturally in a large number of fruits and in honey. Fructose is the sweetest of the common sugars with melting point of 103 – 105 ºC. It occurs as yellowish, white crystals and freely soluble in water. In the present study, the fructose (D-(-)-Fructose) LA grade (HiMedia Laboratories Pvt. Ltd. Mumbai, India) was used as carbon source at 20, 30 and 40 g/l of the medium in our experiments.



Fructose Structure

3.1.3.4 Mannitol $C_6H_{14}O_6$

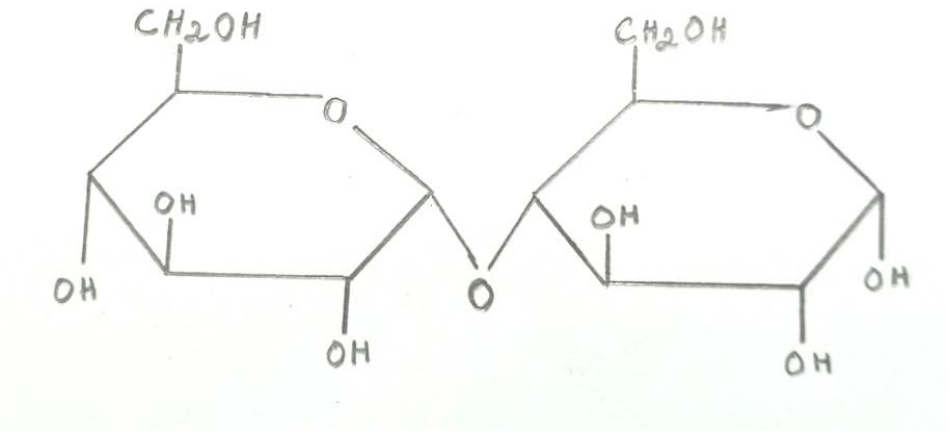
Mannitol is a type of carbohydrate also known as sugar alcohol. It contains about 60 per cent fewer calories than sugar. It's a derivative of monosaccharides mannose. It is a white, crystalline powder or granules, odorless and sweet in taste. It is soluble in water, slightly soluble in alcohols and amines, but insoluble in other organic solvents. It has a high melting point at 165-167ºC. Its molecular weight is 182.172 g/mol and contains 39.6 per cent carbon, 7.77 per cent hydrogen and 52.8 per cent oxygen. In the present study, the mannitol (D-Mannitol) LA grade (HiMedia Laboratories Pvt. Ltd. Mumbai, India) was used as carbon source at 20, 30 and 40 g/l of the medium in our experiments.



Mannitol Structure

3.1.3.5 Maltose $C_{12}H_{22}O_{11}$

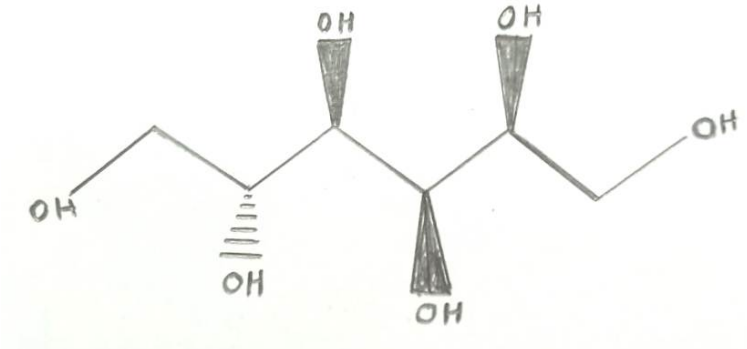
Maltose is the most common reducing disaccharide composed of two molecules of glucose. It is also known as malt sugar found in starch and glycogen. Maltose is obtained by the enzymatic action of diastase (usually obtained from malt extract) on starch. It is colorless crystal having melting point of 102 to 103 ºC, soluble in water, alcohol and insoluble in ether (Arthur and Rose, 1966). In the present study, the maltose (D-(+)-Maltose monohydrate) LA grade (HiMedia Laboratories Pvt. Ltd. Mumbai, India) was used as carbon source at 20, 30 and 40 g/l of the medium.



Maltose Structure

3.1.3.6 Sorbitol $C_6H_{14}O_6$

Sorbitol is a hydrogenated monosaccharide also known as D-glucitol. Like mannitol it is a hexahydric alcohol that is highly soluble in water and can pass through cell membranes. It is converted to fructose by sorbitol-6-phosphate 2-dehydrogenase. Sorbitol is an isomer of mannitol, the two differ only in the orientation of the hydroxyl group on the second carbon. It has a melting point of 94-96ºC and molecular mass at 182.17 g/mol and contains 39.56 per cent carbon, 7.74 per cent hydrogen and 52.70 per cent oxygen. In the present study, the sorbitol (D-(-)-Sorbitol) LA grade (HiMedia Laboratories Pvt. Ltd. Mumbai, India) was used as carbon source at 20, 30 and 40 g/l of the medium in our experiments.



Sorbitol Structure

3.1.3.7 Table sugar ($C_{12}H_{22}O_{11}$)

It is crude sucrose derived by crushing and extraction of sugarcane (Saccharum officinarum). Sugar is hard, white, dry crystals, lumps or powder, sweet taste, odourless, soluble in water and very slightly soluble in alcohol (Arthur and Rose, 1966). Raw cane sugar contains about 96 to 97 per cent sucrose. It has a same molecular formula and structure as sucrose. In the present study, the medium was supplemented with common grade sugar from local market at 20, 30 and 40 g/l of the medium in the experiments.

3.1.3.8 Jaggery

Jaggery is the product obtained on concentrating sugarcane juice with or without prior purification, into a solid or semi solid state. It is also called gur, contains all the constituents of cane juice, some of them having undergone slight changes during boiling. Percentage composition of a typical jaggery is sucrose 65-85 per cent, invert sugar 10-15 per cent, ash 2.5 per cent and moisture 3-6 per cent. It also contains carotene 280 I.U./100 g, nicotinic acid 1.0 mg./100 g, vitamin B1 20 ug/100 g and traces of iron and copper (Anonymous, 1957). In the present study, the medium was supplied with locally made jaggery available at market at 20, 30 and 40 g/l of the medium as carbon source.

3.1.4 Plant Growth regulators

The following plant growth regulators has been used in different experiments at different concentrations on shoot bud induction and rooting medium consisting of – MS Basal medium.

Cytokinins : Benzylaminopurine (BAP)
Auxins : Naphthalene Acetic Acid (NAA)
Indole Butyric Acid (IBA)

3.1.5 Medium

Modified Murashige and Skoog (MS) medium (1962) was used in the

present study. MS media in full strength was used for regeneration of multiple buds (Table 3.1), however half strength MS medium was used in rooting studies. Modification to the medium was done by adding different concentration of carbon sources and other organic additives.

Table 3.1 Compositions of Murashige and Skoog (MS) medium (1962).

Ingredients	Amounts (mg/l)
1. Macro salts	
a) NH_4NO_3	1650
b) KNO_3	1900
c) $CaCl_2.2H_2O$	440
d) $MgSO_4.7H_2O$	370
e) KH_2PO_4	170
2. Micro salts	
a) KI	0.830
b) H_3BO_3	6.200
c) $MnSO_4.4H_2O$	22.300
d) $ZnSO_4.7H_2O$	8.600
e) $Na_2MoO_4.2H_2O$	0.250
f) $CuSO_4.5H_2O$	0.025
g) $CoCl_2.6H_2O$	0.025
3. Fe. EDTA	
a) $FeSO_4.7H_2O$	27.80
b) Na_2. EDTA. $2H_2O$	37.20
4. Organics	
a) Thiamine. HCl	0.10
b) Pyridoxine. HCl	0.50
c) Nicotinic Acid	0.50
d) Glycine	2.00
e) Myo-inositol	100.00
f) Sucrose	30,000.00

- pH = 5.7
- Agar = 0.8%
- Growth regulators, organics and complex organic mixtures were added as per the requirement of experiment.

Table 3.2 Compositions of stock solutions used for the preparation of 1 litre modified MS medium.

Ingredients	Amounts (g/l)
1. Micro stock-I	
a) $ZnSO_4.7H_2O$	1.72
b) H_3BO_3	1.24
c) $MnSO_4.4H_2O$	4.46
d) KI	0.17
2. Micro stock-II	
a) $NaMoO_4.2H_2O$	0.250
b) $CoCl_2.6H_2O$	0.025
c) $CuSO_4.5H_2O$	0.025

Table 3.3 Compositions of Iron stock solution used for the preparation of modified MS medium.

Ingredients	Amounts (g/l)
a) *$FeSO_4.7H_2O$	5.56
b) Na_2. EDTA. $2H_2O$	7.46

*Dissolved in boiling water with constant stirring

Table 3.4 Compositions and amount of different vitamins and other organic stock solutions used for the preparation of modified MS medium for *in vitro* culture of banana cv. Chinichampa (AAB).

Stock	Amounts (mg/100ml)
a) Thiamine HCl	100
b) Nicotinic acid	100
c) Pyridoxine HCl	100
d) Glycine	100

Table 3.5 Compositions and amount of different growth regulators stock solutions used for the preparation of modified MS medium for *in vitro* culture of banana cv. Chinichampa (AAB).

Stock		Amounts (mg/100ml)
a)	IBA	100
b)	NAA	100
c)	6- BAP	100

*All the growth hormones first dissolved in little (1-2 ml.) amount diluted NaOH solution.

3.2 Methodology

The various procedures that were followed for *in vitro* studies of *Musa* cv. Chinichampa (AAB) on modified medium are highlighted below in a step – wise procedure.

3.2.1 Preparation of explants

Healthy and vigorous sword suckers (2-3 months) and immature male floral buds (MFBs) of banana cv. 'Chinichampa (AAB), free from viruses and other diseases were collected and used as a source of explant for the present study. Male floral buds (MFBs) were collected 3-4 weeks after the opening of the bract and brought to the laboratory. The MFBs was reduced in size to 5-6 cm long by removing the outer bracts one by one using sterilized scalpel.

Isolated suckers and male floral buds are washed in diluted Geepol solution (3-4 drops/l), thereafter washed repeatedly in running tap water for 1 hour. Followed by treatment in Bavistin solution (5g/l) for another half an hour with constant stirring. Again kept in running tap water for half an hour to remove all traces of chemicals. Older leaves and extraneous corm tissues were carefully removed with a sterilized sharp stainless knife. Shoot tips containing several leaf bases enclosing the axillary buds and subjacent corm tissues and measuring about 6 to 8 cm in length were isolated. Finally, these shoot tips and

MFBs were kept in a solution of ascorbic acid (1g/100 ml) for about 10 mins to prevent blackening due to oxidation of phenolics compound.

The excised explants are then sterilized in 70% ethyl alcohol for 3 minute under the laminar low cabinet with vigorous shaking, followed by 0.1% Mercury chloride for 1 minute. Finally, shoot tips (2-3cm) and MFBs (1.5-2 cm) size were excised from these sterilized explants and inoculated in culture medium.

3.2.2 Preparation of nutrient media

Media supplemented with different carbon sources at different concentrations (20 g/l, 30 g/l and 40 g/l) were used depending on the purpose of the individual experiment. Stock solutions were prepared for macronutrients, micronutrients, vitamins, iron and growth regulators separately.

3.2.2.1 Preparation of stocks

The stock solutions (10x) were prepared as given below with double distilled water, poured into well stoppered conical flasks and were stored in refrigerator at 4ºC.

Stock A: Micro nutrients – 1000 ml (10x) –Table: 3.2
Stock B: Macro nutrients – 1000 ml (10x) –Table: 3.2
Stock C: Iron – 100 ml (200x) – Table: 3.3
Stock D: Vitamin – 100 ml (50x) –Table: 3.4

3.2.2.2 Preparation of growth regulator stocks

Stock solutions of 6-benzylaminopurine (BAP), IBA and NAA were prepared by dissolving them first in few drops of 1N NaOH and the volume was made up to the required concentration with double distilled water Table: 3.5.

3.2.2.3 Preparation and sterilization of media

The stock solutions were mixed in required proportion along with growth regulators and other vitamins and organic solutions. Different sources of carbon

and concentrations (20g/l, 30g/l and 40g/l) along with Myo-inositol 100 mg/l, L-Tyrosine 100mg/l, Adenine Sulphate 80mg/l were added fresh to the medium and the volume was made up. The pH of the medium was adjusted between 5.6-5.8 by using either 0.1 N HCl or NaOH with the help of a digital pH meter. The volume was finally adjusted by adding double distilled water and agar 7 g/l was added and dissolved by gentle heating up to 90^{o}C, with frequent stirring to ensure uniform heating and to avoid boiling or frothing. About 60 ml of medium were dispensed into sterilized glass culture bottles (12cm x 6cm) and plugged with plastic corks and sealed with tape to prevent any loss of moisture or contamination during handling and autoclaving. The media was autoclaved at 121^{o}C at 16 *psi* for 15 minutes and then allowed to cool to room temperature and stored in culture rooms until further use.

3.2.3 Physical factors

All the plant tissue culture experiments were conducted under defined conditions of the culture room maintained at 25±2°C and 60-70% RH, under alternate 12 hrs light (intensity of 2000 lux) and 12 hrs dark cycle. Further light intensity and duration was enhanced up to 16 hrs (3000 lux intensity), during subsequent culture. The *in vitro* culture operations were carried out aseptically under a laminar airflow chamber. Borosilicate culture bottles, glasswares and analytical grade chemicals were used.

Ingredients/ Stock solution	Operations
	Poured 500 ml distilled water into 1 litre capacity beaker with magnetic stirring bar
Macro salts →	Weighed, added and dissolved all macro salts ingredients individually
Iron stock & Micro stock-I →	Added 5 ml each
Micro stock-II →	Added 1 ml
Glycine stock →	Added 2 ml
Nicotinic acid & Pyridoxine HCl →	Added 0.5 ml each
Thiamine HCl →	Added 0.1 ml
M-inositol & L-Tyrosine →	Weighed, added and dissolved 0.1 g
Adenine Sulphate →	Weighed, added and dissolved 0.08 g ↓ Added aliquot of Cytokinin and Auxin stock ↓ Weighed, added and dissolved carbon source ↓ Poured into graduated cylinder and adjusted the volume to 990 ml ↓ Adjusted pH to 5.6- 5.7 and volume made up to 1 litre ↓ Poured into a stainless steel container, heated to a near boiling point, added 7 g Agar powder and dissolved ↓ Dispensed into culture bottles ↓ Autoclaved at 15 p.s.i. for 15 minutes ↓ Stored in inoculation room

Fig 3.1 Schematic diagram for preparation of 1 litre Modified MS medium

Table 3.6 Compositions and amount of stock solutions used in the preparation of 1 (one) litre modified MS medium for *in vitro* culture of banana cv. Chinichampa (AAB).

Constituents	Quantities
A. Macro salts	
NH_4NO_3	1.65 g l^{-1}
KNO_3	1.90 g l^{-1}
$CaCl_2.2H_2O$	0.44 g l^{-1}
$MgSO_4.7H_2O$	0.37 g l^{-1}
KH_2PO_4	0.17 g l^{-1}
$NaH_2PO_4.H_2O$	0.09 g l^{-1}
B. Minor stock-I	5 ml l^{-1}
C. Micro stock-II	1 ml l^{-1}
D. Iron stock	5 ml l^{-1}
E. Vitamins, amino acids and other organic supplements	
Thiamine. HCl	0.1 ml l^{-1}
Pyridoxine. HCl	0.5 ml l^{-1}
Nicotinic Acid	0.5 ml l^{-1}
Glycine	2 ml l^{-1}
Myo-inositol	0.1 g l^{-1}
L-Tyrosine	0.1 g l^{-1}
Adenine Sulphate	0.08 g l^{-1}
F. Growth regulators	
6- BAP	(4mg l^{-1})
TDZ	(0.1mg l^{-1})
IBA	(2mg l^{-1})
NAA	(0.5mg l^{-1})
G. Sugar	20/30/40 g l^{-1}
H. Agar	7 g l^{-1}
I. pH (maintained)	5.6 - 5.7

3.2.4 Experimental details

3.2.4.1 Multiple culture establishment and shoot proliferation

Shoot tips (2-3cm) and MFBs (1.5-2cm) explants were cultured on MS medium supplemented with various concentrations of carbon sources (Table: 3.7) along with 4 mg/l BAP and 0.1mg/l TDZ.

Table 3.7 Compositions of MS media with various concentrations of carbon sources for *in vitro* culture establishment and shoot proliferation of banana explants (shoot tip & MFBs) cv. Chinichampa (AAB)

Treatment codes	Carbon sources
T_1 - SPM S	Sucrose 30 g/l
T_2 - SPM G-2	Glucose 20 g/l
T_3 - SPM G-3	Glucose 30 g/l
T_4 - SPM G-4	Glucose 40 g/l
T_5 - SPM F-2	Fructose 20 g/l
T_6 - SPM F-3	Fructose 30 g/l
T_7 - SPM F-4	Fructose 40 g/l
T_8 - SPM Man-2	Mannitol 20 g/l
T_9 - SPM Man-3	Mannitol 30 g/l
T_{10} - SPM Man-4	Mannitol 40 g/l
T_{11} - SPM Mal-2	Maltose 20 g/l
T_{12} - SPM Mal-3	Maltose 30 g/l
T_{13} - SPM Mal-4	Maltose 40 g/l
T_{14} - SPM Sor-2	Sorbitol 20 g/l
T_{15} - SPM Sor-3	Sorbitol 30 g/l
T_{16} - SPM Sor-4	Sorbitol 40 g/l
T_{17} - SPM TS-2	Table sugar 20 g/l
T_{18} - SPM TS-3	Table sugar 30 g/l
T_{19} - SPM TS-4	Table sugar 40 g/l
T_{20} - SPM J-2	Jaggery 20 g/l
T_{21} - SPM J-3	Jaggery 30 g/l
T_{22} - SPM J-4	Jaggery 40 g/l

*SPM = Shoot Proliferation Medium

The days required for greening, swelling, culture established percentage was recorded. After 4 weeks of culture the days required for multiple buds initiation, multiple buds culture percentage, number of multiple buds produced per explants and length of multiple buds after 30 days were recorded in both the experiments for Shoot tips and MFBs.

3.2.4.2 Regeneration and root initiation

Uniformed multiple shoots obtained from shoot tip and MFBs explants through repeated subculturing were excised and placed on modified MS basal media for shoot elongation. Well elongated shoots were transferred to half

strength basal MS medium supplemented with various concentrations of carbon sources (Table: 3.8) along with 2mg/l IBA and 0.5 mg/l NAA to promote rooting *in vitro.*

Table 3.8 Compositions of MS media with various concentrations of carbon sources for *in vitro* rooting of banana explants (shoot tip & MFBs) cv. Chinichampa (AAB).

Treatment codes	Carbon sources
T_1 - RIM S	Sucrose 30 g/l
T_2 - RIM G-2	Glucose 20 g/l
T_3 - RIM G-3	Glucose 30 g/l
T_4 - RIM G-4	Glucose 40 g/l
T_5 - RIM F-2	Fructose 20 g/l
T_6 - RIM F-3	Fructose 30 g/l
T_7 - RIM F-4	Fructose 40 g/l
T_8 - RIM Man-2	Mannitol 20 g/l
T_9 - RIM Man-3	Mannitol 30 g/l
T_{10} - RIM Man-4	Mannitol 40 g/l
T_{11} - RIM Mal-2	Maltose 20 g/l
T_{12} - RIM Mal-3	Maltose 30 g/l
T_{13} - RIM Mal-4	Maltose 40 g/l
T_{14} - RIM Sor-2	Sorbitol 20 g/l
T_{15} - RIM Sor-3	Sorbitol 30 g/l
T_{16} - RIM Sor-4	Sorbitol 40 g/l
T_{17} - RIM TS-2	Table sugar 20 g/l
T_{18} - RIM TS-3	Table sugar 30 g/l
T_{19} - RIM TS-4	Table sugar 40 g/l
T_{20} - RIM J-2	Jaggery 20 g/l
T_{21} - RIM J-3	Jaggery 30 g/l
T_{22} - RIM J-4	Jaggery 40 g/l

*RIM = Root Induction Medium

The days required for rooting and rooting percentage were recorded. After 4 weeks of culture the number of roots per shoot, length of longest roots, average length of shoots and number of leaves per plantlets were recorded for both the experiments using shoot tips and MFBs.

3.3 Growth measurements

The effect of different carbon sources in MS medium on the growth of different explants in culture was analyzed by taking the following parameters. The observations were recorded on shoot characters and root characters.

3.3.1. Shoot characters

Days required to greening and swelling and multiple buds initiation were recorded by visually inspecting the inoculated cultures for changes in colour, swelling and the day initiation were recorded at the base of the explants. Assessments of other shoot characters are made based on the following formula:

a) Culture established (%)

It was calculated using the following formula:

$$\text{Culture established (\%)} = \frac{\text{Total no. of explants survived}}{\text{Total no. of explants inoculated}} \times 100$$

b) Culture showing multiple buds (%)

It was calculated using the following formula:

$$\text{Culture showing multiple buds (\%)} = \frac{\text{Total no. of explants culture showing multiple}}{\text{No. of explants inoculated}} \times 100$$

c) No. of multiple buds produced per explants

The number of multiple buds produced by each explants was recorded and mean was calculated by using following formula:

$$\text{No. of multiple buds produced per explants} = \frac{\text{Total no. of multiple buds produced}}{\text{Total no. of explants inoculated}}$$

d) Length of multiple buds (cm) after 30 days

Length of all the multiple buds produced per explant was measured by using iron graduated scale from base of collar region to the top of newly emerged leaf, their mean was calculated and expressed in centimeters.

3.3.2 Root characters

The time taken for root initiation was recorded in days when the micro shoots starts initiating roots after inoculated in rooting medium. Other observations recorded during root initiation are described below.

a) Rooting percentage (%)

It was calculated using following formula:

$$\text{Rooting percentage} = \frac{\text{Total no. of micro shoot rooted}}{\text{Total no. of micro shoots inoculated}} \times 100$$

b) No. of roots/ shoot

The total number of primary roots formed per microshoots was recorded and average was worked out.

c) Length of longest roots (cm) after 30 days

The length of the longest primary root was measured from base of the roots and average was expressed in centimeters.

d) Average length of shoot (cm) after 30 days

The length of all the rooted shoots were measured from the base of the collar region to tip of the longest leaves and expressed in centimeters.

e) No. of leaves/ plantlets

The total numbers of leaves formed per rooted shoots were recorded and average was worked out.

3.4 Statistical analysis

The present studies were conducted in the laboratory under *in vitro* conditions. Completely Randomized Design (CRD) was employed for all the experiments. All the experiments were replicated thrice. There were 22 treatment combinations for each experiment with ten (10) numbers of explants per treatment. The effects of treatments were tested by Analysis of Variance (ANOVA); differences among the treatment means were tested by Duncan's Multiple Range Test (DMRT), (Duncan, 1955) at $P \leq 0.05$.

3.5 Differential cost analysis

In the present study, different carbon sources *(i.e.* sucrose, glucose, fructose, mannitol, sorbitol, maltose, table sugar and jaggery) were experimented and the differential cost of production for 1000 plantlets using different carbon sources was worked out.

A) Isolation of sword suckers

B) Shoot tip ready for surface sterilization (10-15 cm)

C) Isolation of male floral buds (MFBs)

D) MFB ready for surface sterilization (5-6 cm)

Plate 1 Different stages for preparation of explants for *in vitro* establishment through shoot tip and male floral buds (MFBs) of *Musa* cv. Chinichampa (AAB)

A) Treatment of explants in Bavistin

B) Treatment of explants in 70% IPA

C) Treatment of explants in mercury chloride (0.1%)

D) Excision of shoot tip (2-3cm) in SPM medium

E) Excision of MFBs (1.5-2cm) in SPM medium

Plate 2 Different stages for preparation of explants for *in vitro* establishment through shoot tip and male floral buds (MFBs) of *Musa* cv. Chinichampa (AAB)

CHAPTER IV

RESULTS AND DISCUSSION

RESULTS AND DISCUSSION

This chapter summarizes the entire findings recorded and discussed parameter wise with appropriate justification and reasons. The present study was carried out to determine the responses of various concentrations of carbon sources on *in vitro* culture establishment, proliferation and rooting of banana explants cv. Chinichampa (AAB) from shoot tips and male floral bud (MFBs), subculture studies and hardening of the regenerated plantlets with differential cost of production for 1000 plantlets by different carbon sources. The detailed data collected during the study and the results have been presented in this chapter supported by respective tables and figures.

4.1 Experiment – 1

The present study was conducted to identify the best sources of carbon for *in vitro* culture establishment and shoot proliferation of banana explants cv. Chinichampa (AAB) through shoot tip.

4.1.1 Days to greening

Days require to greening was recorded by visually inspecting the inoculated cultures for changes in colour. The data obtained with regard to days to greening is presented in table 4.1. There was significant difference in all the treatments for days taken for greening of shoot tips culture. Minimum day required for greening was noted in T_1 (4.63 days) followed by T_4 (5.34 days) which was significantly at par with T_3 (5.77 days) and T_{18} (5.93 days). While the longest days required for greening was recorded in T_{20} (16.33 days).

Shoots tips of banana cv. Chinichampa micropropagated on different carbon sources gave different performance in their growth and proliferation. Among all the carbon sources, sucrose T_1 (4.63 days) was found best for the shoot tips proliferation. Similar findings have been reported in

micropropagation of Brahmi (Preeti *et al*., 2017), Cork Oak (Romano *et al*., 1995) and *Kaemferi galangal* (Fatima *et al*., 2000) where sucrose was found best choice as carbon source. This may be due to its efficient uptake across the plasma membrane (Borkowska and Szezebra, 1991). Lee *et al*. (2012) also reported that sucrose is commonly used at the concentration of 20 and 30 g/l as carbon source in tissue culture media. One of the important properties of sucrose on growth of explants under *in vitro* condition is because of its wide use as transport molecule in plant system, high solubility in water, electrical neutrality, and its apparent lack of inhibitory effect on majority of biochemical processes (Smith, 1995).

4.1.2 Days to swelling

Days require to swelling was recorded by visually inspecting the inoculated cultures for swelling and the day of initiation were recorded. The data regarding days require for swelling is showed in table 4.1 and displayed graphically in figure 4.2. The minimum number of days for initiation of swelling was recorded under T_1 (14.00 days) which was statistically at par with both T_{18} (14.58 days) and T_8 (14.62 days). While the maximum number of days for initiation of swelling was observed in T_{16} (29.00 days) which was at par with T_{21} (28.67 days).

Different carbohydrates tends to dominate the morphogenesis of tissues by serving as the energy source and also by changing the osmotic potential of the culture medium, these in turn changes the cell wall properties and composition followed by subsequent modification in morphogenesis (Pritchard *et al*., 1991). There are reports of different carbon sources affecting the *in vitro* regeneration of different species (Fuentes *et al*., 2000). Preeti *et al*. (2017) found that increase in concentration of sucrose from 0.5 to 5% caused increased in caulogenesis in Brahmi. But in contrast Mitchel *et al*. (2008) and Sujana and Naidu (2011) reported that 4% glucose and fructose were found

optimum for caulogenesis in *Gossypium hirsutum* and *Mentha piperata* respectively. However in apple stock M9 and M26 the best morphogenic response was recorded in sorbitol among the different carbohydrates sources trialed *in vitro* (Mehwish *et al*., 2009).

4.1.3 Culture established (%)

The data regarding culture established (%) has been depicted in table 4.1 and figure 4.3. The highest percentage of culture establishment was recorded in T_1 (46.67%). It was observed that there was no culture establishment in T_{11}, T_{20}, and T_{22}. The lowest percentage of culture establishment was recorded in $T_{13,}$ T_{14} and T_{16} (6.67%).

In plant tissue culture, carbohydrate serves as the main energy source in the culture medium and supplement or modifies the osmotic pressure developed in the plant cells (Huh *et al*., 2016). Many researchers consider sucrose as the most important carbohydrate for plant tissue culture, as it is the most available carbohydrate in phloem sap of plant cells and participate in various morphogenesis of tissues (Gibson, 2000). Sucrose is known to act as a balanced source of energy for cell enlargement and thus participate directly in glycolytic and pentose phosphate pathway (Zha *et al*., 2007). In most commercial tissue culture protocols, sucrose is mostly used source of sugar as it is readily assimilated nutrient source and quickly transformed into available energy (Saraswathi *et al*., 2016). The failure of culture establishment in jaggery supplemented in shoot proliferation media may be due inhibitors present or formed during autoclaving. It may also be due to adverse effect of jaggery on shoot and root growth as opined by Joshi *et al*. (2009) in micropropagation of *Wrightia tomentosa*.

4.1.4 Days required for multiple buds initiation

In this investigation, the response of different sources of carbon on MS medium were tested for effective *in vitro* shoot proliferation in banana cv.

Chinichampa (AAB) shoot tip culture. The data for days required for multiple buds initiation were recorded and presented in table 4.1 and graphically represented in figure 4.4. Multiple buds initiation started after three weeks of inoculation in most of the medium tested. It was evident from the table that T_1 (24.28 days) required minimum days for multiple buds initiation followed by T_{18} (25.33 days) and T_3 (27.17 days). While the treatment T_7 (38.00 days) required maximum number of days for multiple buds initiation. T_{10} (37.67 days) showed similar results with treatment T_7 in days required for multiple buds initiation. It was also observed that treatments T_5, T_{11}, T_{14}, T_{20}, T_{21} and T_{22} did not show any multiple buds initiation.

It is already known that the addition of carbohydrate into the culture medium is vital for plantlet growth and regeneration. In *in vitro* culture photosynthesis is limited; therefore, it requires an external energy source in the form of carbohydrate which is provided by adding sucrose as a carbon sources (Kozai *et al*., 1997). Sucrose is highly preferred carbon source as it is a non reducing sugar and acts as a transporter of essential molecules that has high water solubility and easily permeates through plasma membrane (Baskaran and Jayalabalan, 2005; Javed and Ikram, 2008).

4.1.5 Culture showing multiple buds (%)

The culture showing multiple buds were recorded and expressed in percent as shown in table 4.1 and figure 4.5 It was evident from the table 4.1 that the maximum multiple buds was recorded in T_1 (36.67%) and it was tailed by T_{17} (20.00%) and T_3 (16.67%). Equal percentage (3.34%) of culture showing multiple buds were recorded in T_7, T_{10}, T_{12}, T_{13}, T_{15}, T_{16} and T_{19}. Experimental results also revealed that there was no multiple buds initiation in treatments T_5, T_{11}, T_{14}, T_{20}, T_{21} and T_{22}. Jain *et al.* (1997) has also reported that the inability to fully utilize some of the carbon sources by *in vitro* plantlets could be due to either reduced uptake or absence or insufficient degrading

enzyme activities in some plant species.

4.1.6 Number of multiple buds produced per explant

The number of multiple buds produced by each explants was recorded and expressed in mean value. The observations and findings are presented in table 4.1 and figure 4.6. The highest number of multiple buds was produced by T_{18} (4.00) followed by T_7 (3.33) which were statistically at par with T_{17} (3.33), T_1 (3.17), and T_9 (3.00). The lowest number of multiple buds was recorded in T8 (2.00) which were also at par with T_2 (2.17) and T_{19} (2.17).

Banana *in vitro* cultured on various carbon sources showed varying effects in their growth and morphogenesis which might be due to the difference in reactivity of tissues to the furfural or hydroxyl furfural formed during autoclaving of the media (Hsiao and Borman, 1989). The present findings are also similar with the findings of Saraswathi *et al*. (2016) where 3% table sugar resulted in maximum number of buds per shoots inoculated in Udhayam banana. Table sugar contains 96-97% sucrose (Tyagi *et al*., 2007) as compared with 99.98% sucrose in laboratory grade sucrose which might be one of the reasons it performs better in *in vitro*. Goel *et al*. (2007) also observed better performance with ordinary table sugar in micropropagation of *Rauwolfia serpentina*. In contrast to the present findings, Prabhuling and Sathyanarayan (2017) and Madhulata *et al*. (2006) reported maximum number of multiple buds production with sucrose as carbon source in *in vitro* propagation of Grand Naine and Nendran respectively.

4.1.7 Length of multiple buds (cm) after 30 days

Length of all the multiple buds produced per explant was measured by using iron graduated scale from base of collar region to the top of newly emerged leaf; their mean was calculated and expressed in centimeters. The data and figures have been presented in table 4.1 and figure 4.7. The results revealed that the maximum length of multiple buds was observed in T_1 (3.03 cm) which

were at par with T_{18} (3.00 cm). Treatments like T_4 (2.35 cm), T_9 (2.35 cm) and T_{10} (2.30 cm) were all statistically at par. The shortest length of multiple buds was found in T_{13} (1.80 cm).

In general, sucrose is most commonly used in *in vitro* culture media to induce adventitious buds. Similar finding has been reported by Memon *et al*. (2019) in Basrai banana where 30 g/l sucrose results in maximum length of buds. The same result was replicated by Madhulata *et al*. (2006) in Nendran banana. Jalil *et al*. (2003) also reported that the concentrations of sucrose at 30 g/l and 40 g/l were advantages for clonal propagation of *Musa* Spp. resulting in maximum growth parameters and number of suckers evaluated.

Table 4.1 Effect of modified MS medium supplemented with various concentrations of carbon sources on *in vitro* culture establishment and shoot proliferation of banana explants (shoot tip) cv. Chinichampa (AAB)

Treatments	Days to greening	Days to swelling	Culture established (%)	Days required for multiple buds initiation	Culture showing multiple buds (%)	Number of multiple buds produced per Explants	Length of multiple buds (cm) after 30 days
T_1: SPM S	4.63^{i}	14.00^{e}	46.67	24.28^{h}	36.67	3.17^{ab}	3.03^{a}
T_2: SPM G2	6.64^{gh}	16.73^{de}	26.67	31.00^{e}	13.34	2.17^{c}	2.20^{c}
T_3: SPM G3	5.77^{hi}	15.00^{e}	30.00	27.17^{fg}	16.67	2.83^{bc}	2.07^{cd}
T_4: SPM G4	5.34^{hi}	15.11^{e}	30.00	34.00^{cd}	10.00	2.67^{bc}	2.35^{bc}
T_5: SPM F2	8.75^{ef}	19.95^{cd}	20.00	0.00^{i}	0.00	0.00^{d}	0.00^{e}
T_6: SPM F3	9.11^{ef}	19.11^{cd}	20.00	36.00^{ab}	10.00	2.50^{bc}	2.24^{c}
T_7: SPM F4	7.58^{fg}	18.89^{cd}	20.00	38.00^{a}	3.34	3.33^{ab}	2.14^{cd}
T_8: SPM Man-2	10.00^{e}	21.00^{c}	10.00	34.33^{cd}	6.67	2.00^{c}	2.22^{c}
T_9: SPM Man-3	9.78^{e}	19.61^{cd}	20.00	34.33^{cd}	10.00	3.00^{ab}	2.35^{bc}
T_{10}: SPM Man-4	8.75^{ef}	18.22^{d}	13.34	37.67^{a}	3.34	2.33^{bc}	2.30^{bc}
T_{11}: SPM Mal-2	0.00^{j}	0.00^{f}	0.00	0.00^{i}	0.00	0.00^{d}	0.00^{e}
T_{12}: SPM Mal-3	12.83^{cd}	21.17^{cd}	10.00	32.33^{de}	3.34	2.50^{bc}	2.10^{cd}
T_{13}: SPM Mal-4	13.67^{bc}	22.67^{bc}	3.34	37.00^{ab}	3.34	2.67^{bc}	1.80^{d}
T_{14}: SPM Sor-2	11.45^{d}	23.67^{bc}	3.34	0.00^{i}	0.00	0.00^{d}	0.00^{e}
T_{15}: SPM Sor-3	14.17^{bc}	27.33^{ab}	6.67	35.00^{bc}	3.34	2.33^{bc}	2.27^{bc}
T_{16}: SPM Sor-4	15.00^{ab}	29.00^{a}	3.34	35.00^{bc}	3.34	2.67^{bc}	2.38^{bc}
T_{17}: SPM TS-2	6.20^{gh}	15.14^{e}	30.00	27.67^{f}	20.00	3.33^{ab}	2.98^{a}
T_{18}: SPM TS-3	5.93^{hi}	14.58^{e}	30.00	25.33^{gh}	13.34	4.00^{a}	3.00^{a}
T_{19}: SPM TS-4	7.67^{fg}	15.50^{e}	10.00	30.67^{e}	3.34	2.17^{c}	2.62^{b}
T_{20}: SPM J-2	16.33^{a}	0.00^{f}	0.00	0.00^{i}	0.00	0.00^{d}	0.00^{e}
T_{21}: SPM J-3	14.67^{b}	28.67^{a}	6.67	0.00^{i}	0.00	0.00^{d}	0.00^{e}
T_{22}: SPM J-4	0.00^{j}	0.00^{f}	0.00	0.00^{i}	0.00	0.00^{d}	0.00^{e}
SEm±	*0.49*	*1.63*	-	*0.71*	-	*0.33*	*0.11*
CD (P=0.05)	*1.40*	*4.64*	-	*2.01*	-	*0.93*	*0.32*

*Means with different superscript letters within column are significantly different from each other by DMRT (*p=0.05*)

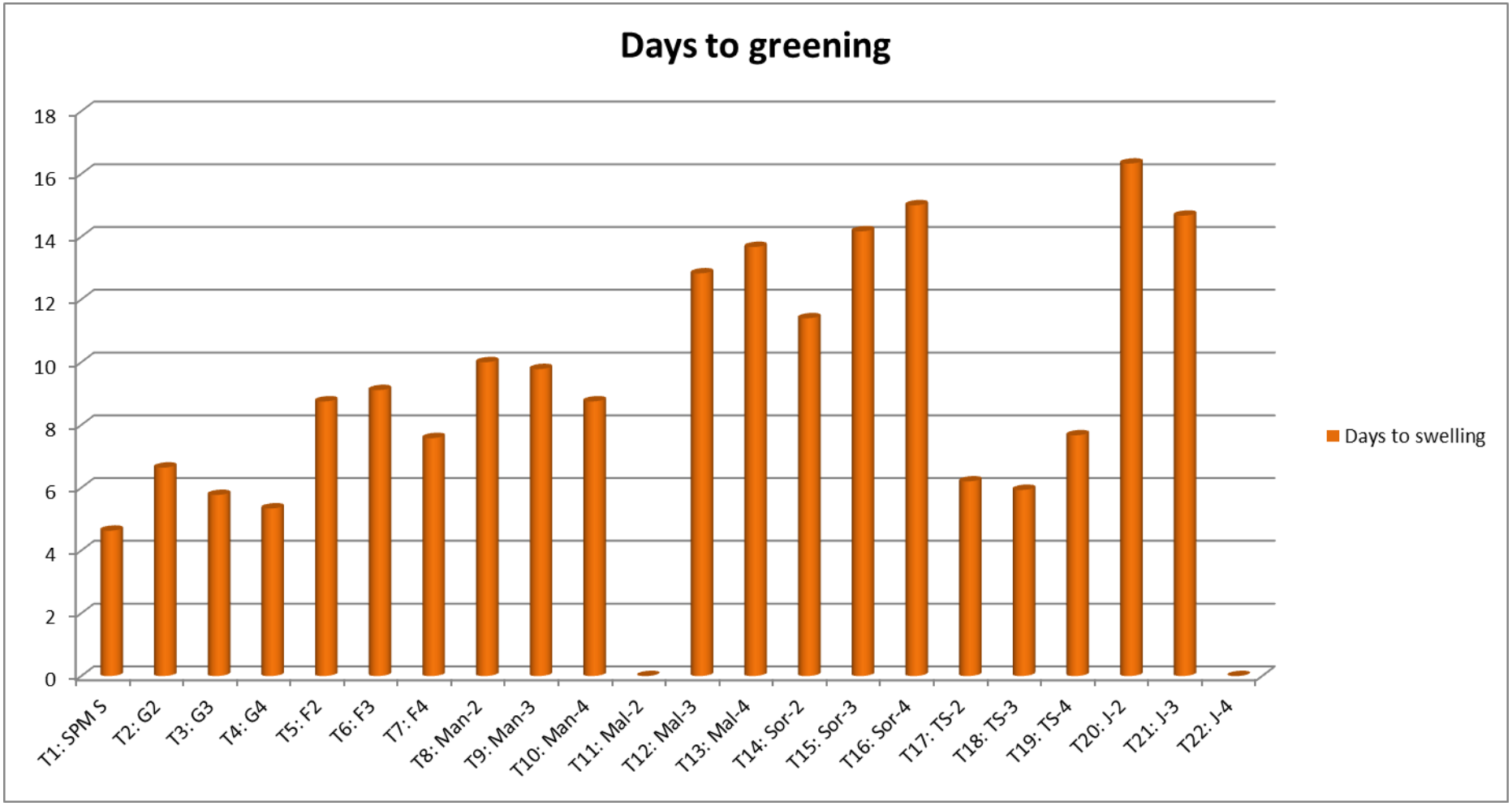


Figure 4.1 Effect of modified MS medium supplemented with various concentrations of carbon sources on days to greening of banana explants (shoot tip) cv. Chinichampa (AAB)

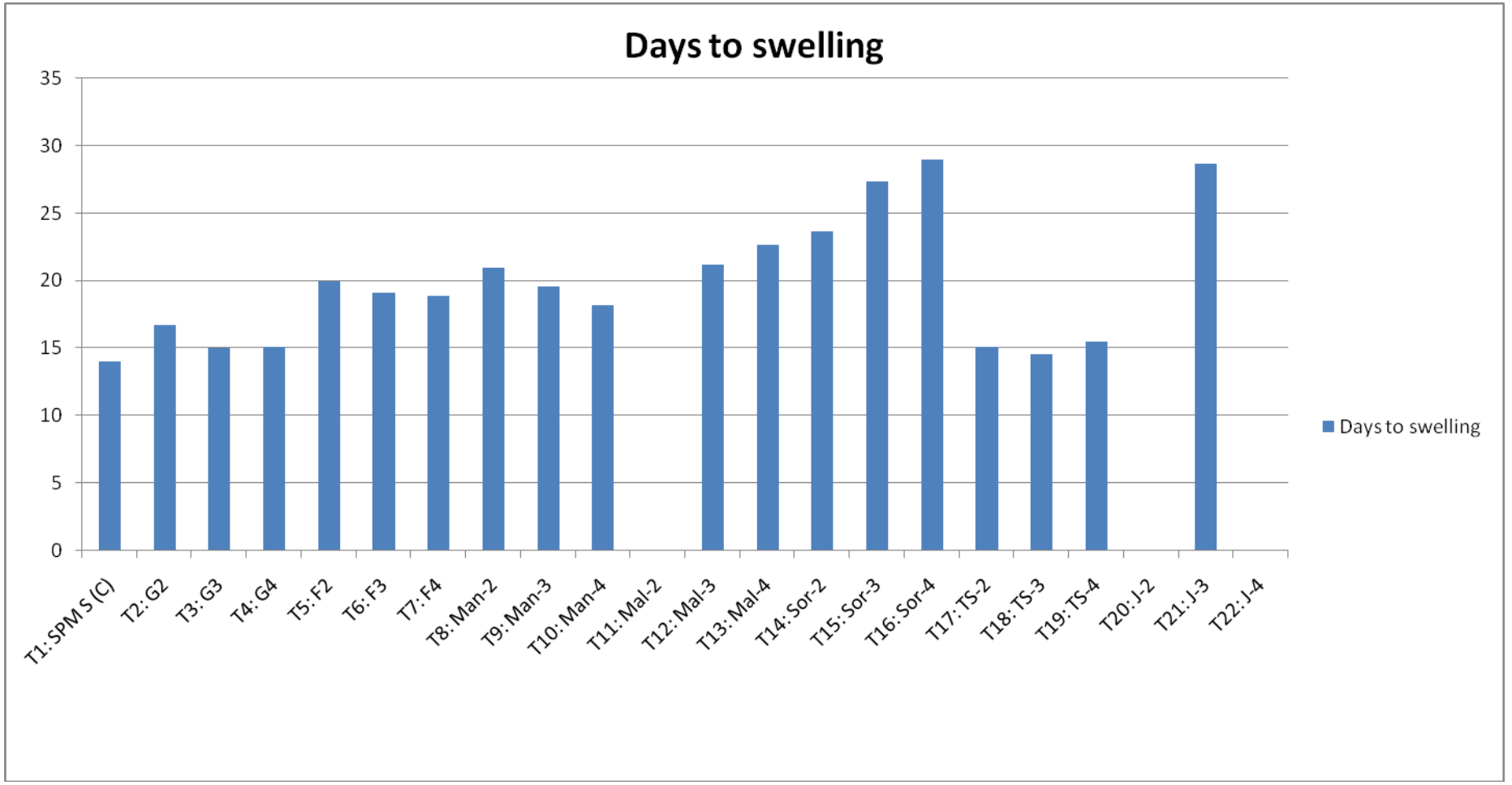


Figure 4.2 Effect of modified MS medium supplemented with various concentrations of carbon sources on days to swelling of banana explants (shoot tip) cv. Chinichampa (AAB)

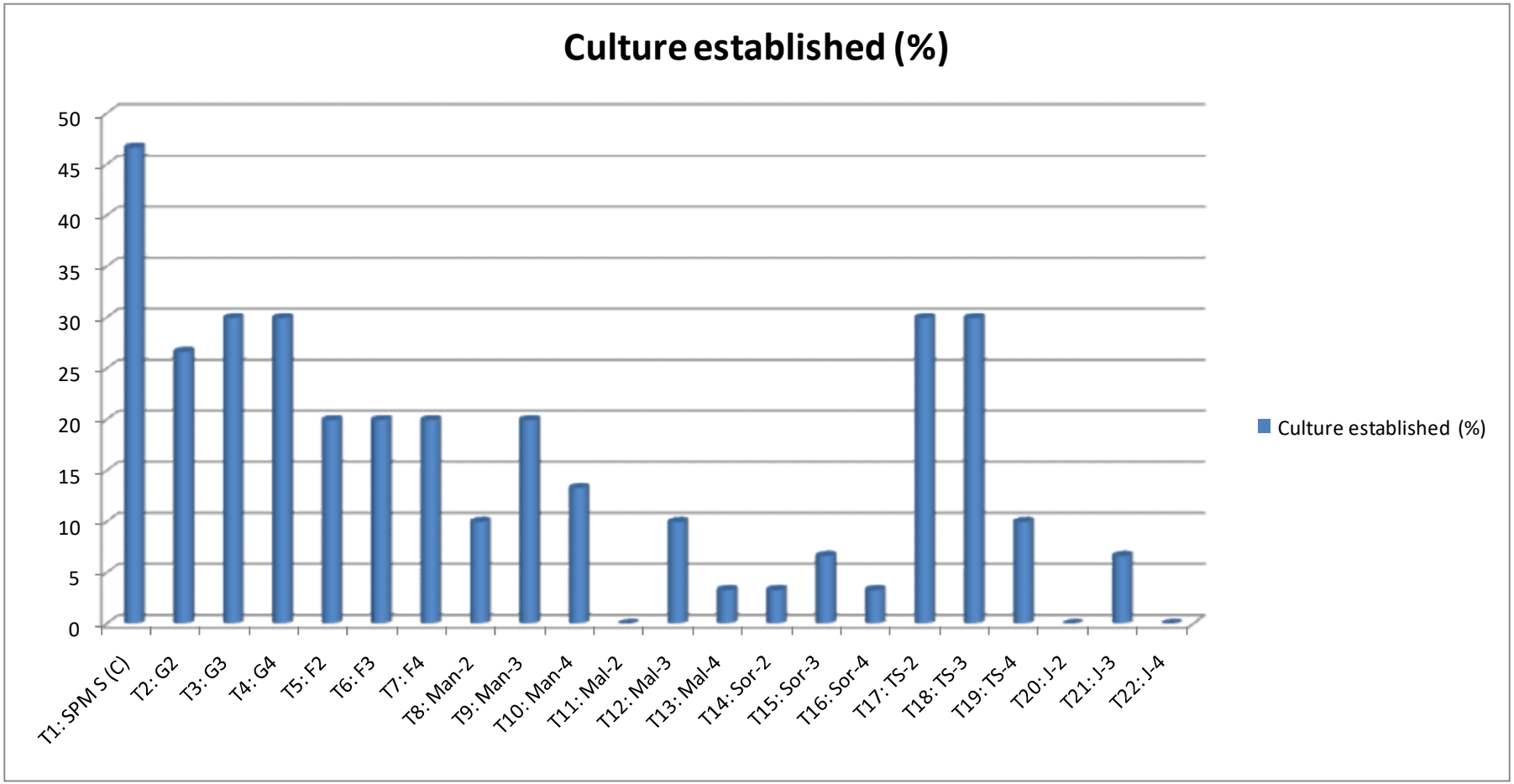


Figure 4.3 Effect of modified MS medium supplemented with various concentrations of carbon sources on culture established (%) of banana explants (shoot tip) cv. Chinichampa (AAB)

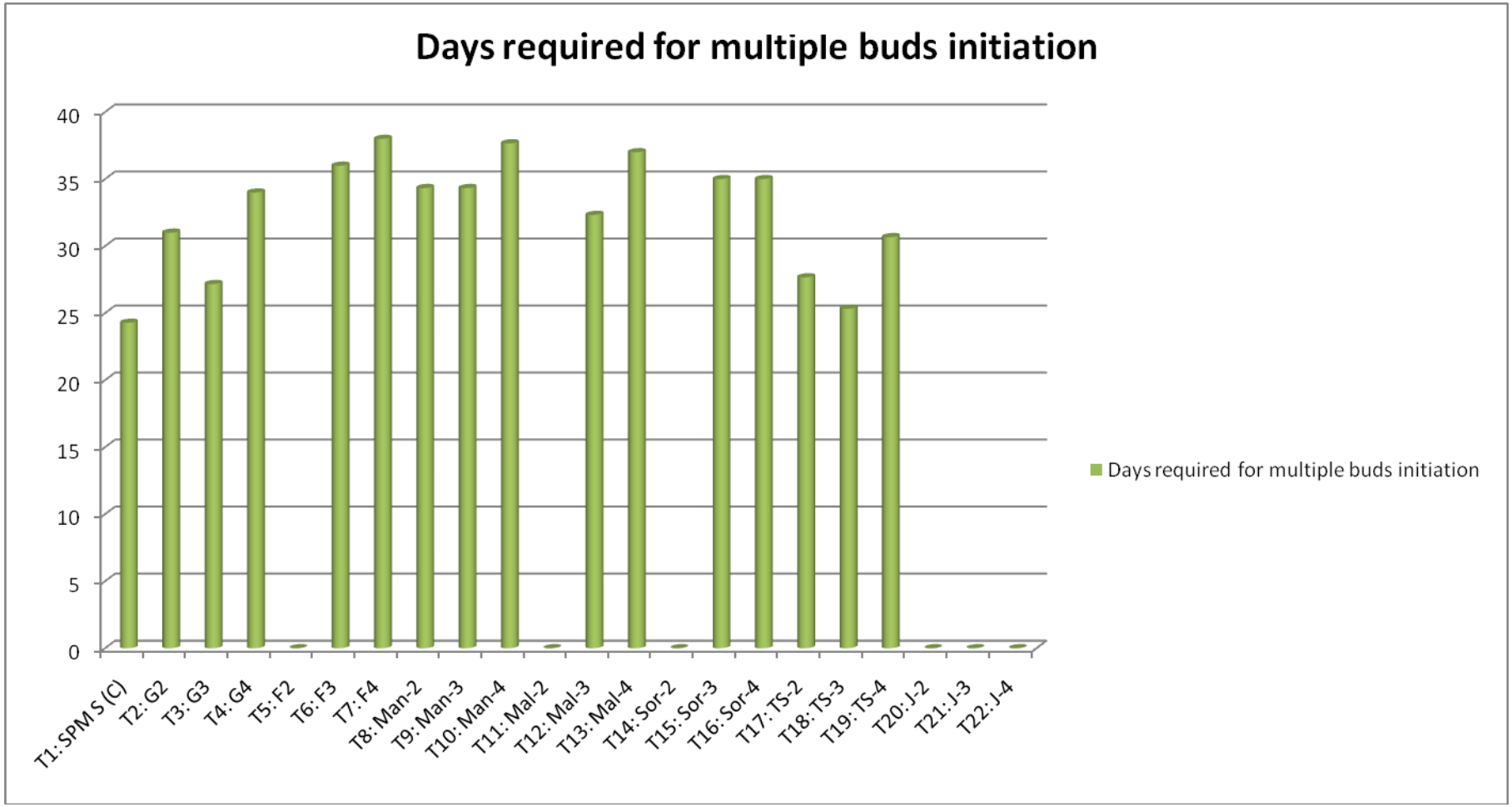


Figure 4.4 Effect of modified MS medium supplemented with various concentrations of carbon sources on days required for multiple buds initiation of banana explants (shoot tip) cv. Chinichampa (AAB)

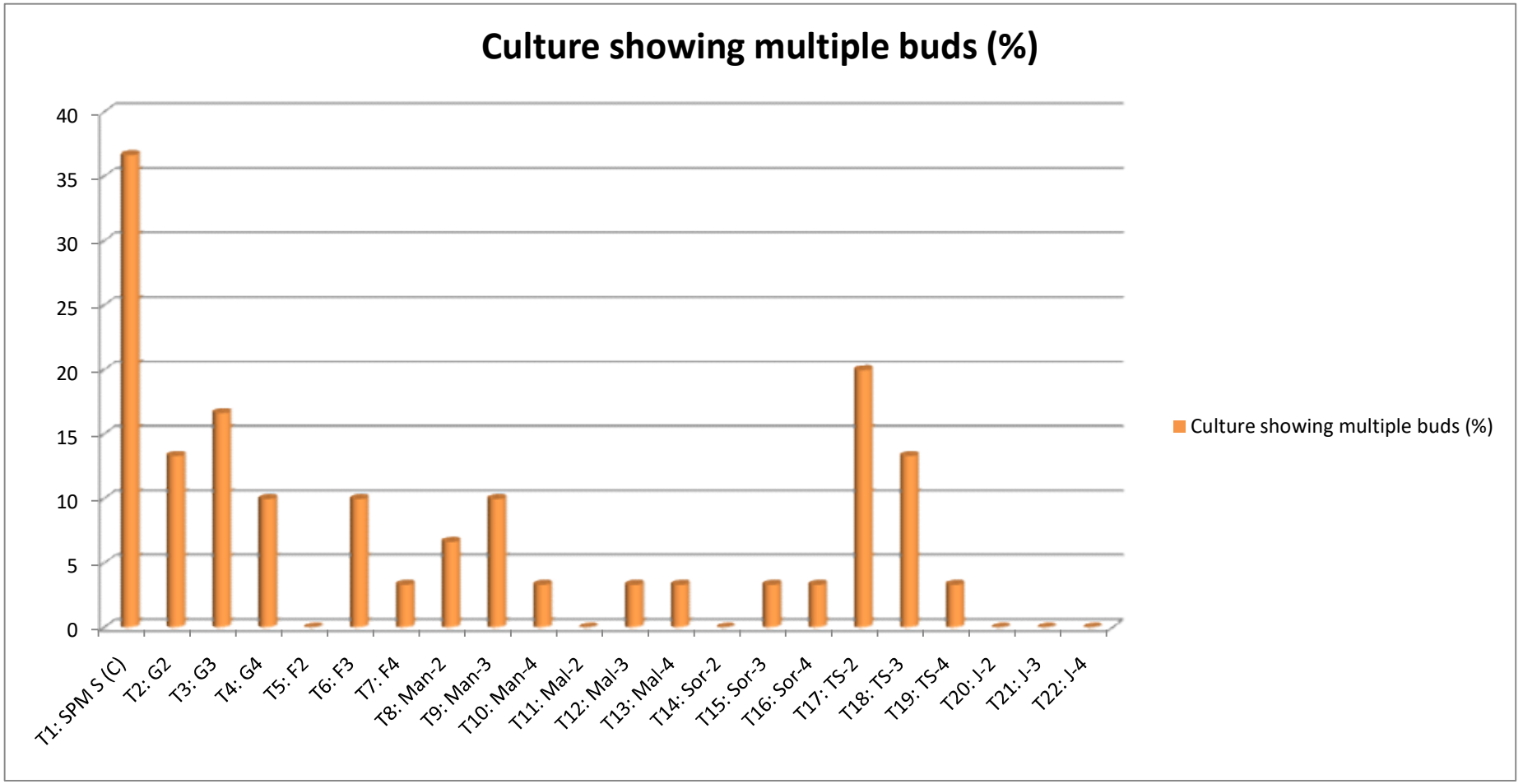


Figure 4.5 Effect of modified MS medium supplemented with various concentrations of carbon sources on culture showing multiple buds (%) of banana explants (shoot tip) cv. Chinichampa (AAB)

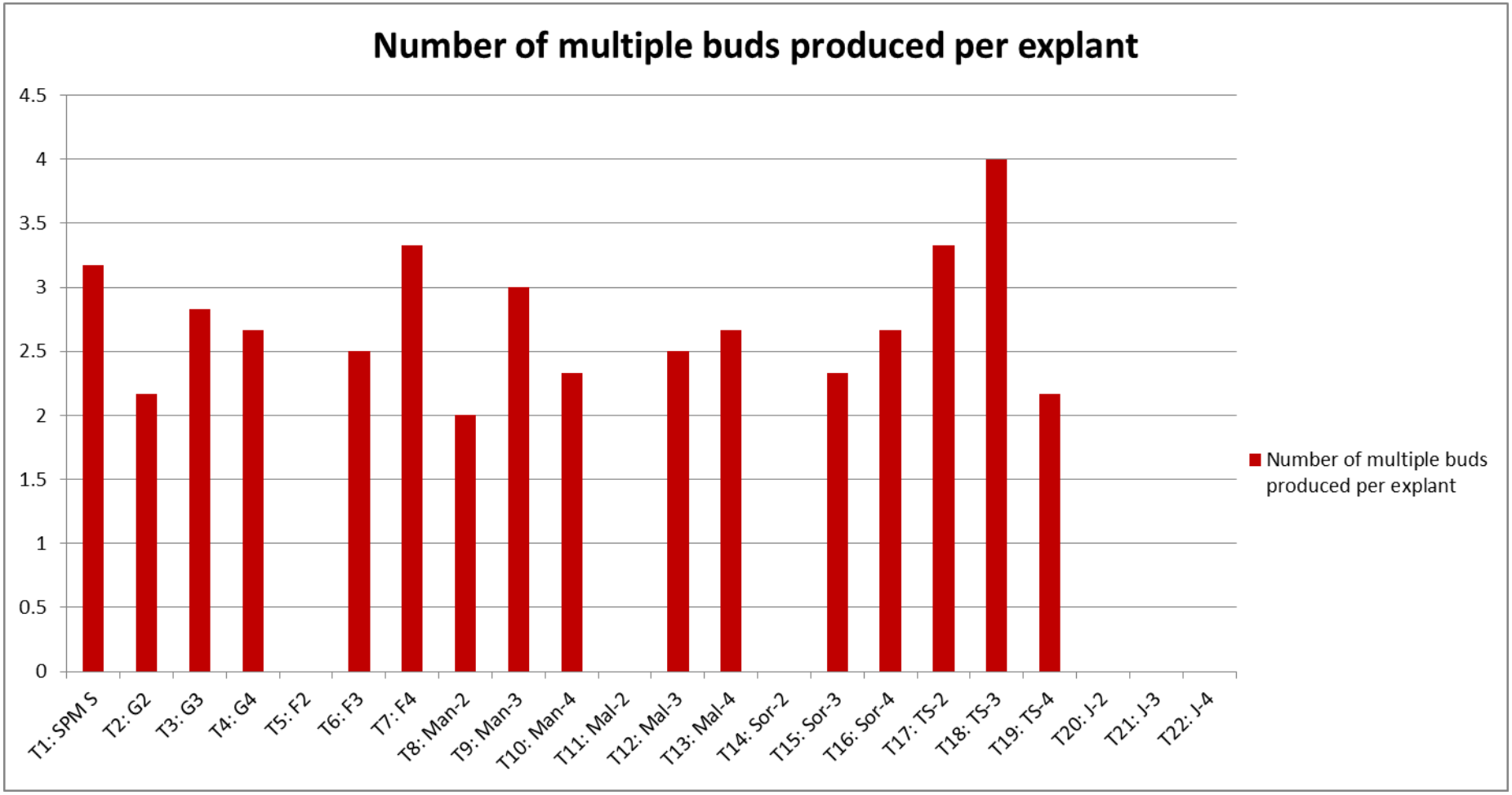


Figure 4.6 Effect of modified MS medium supplemented with various concentrations of carbon sources on number of multiple buds produced per explant of banana explants (shoot tip) cv. Chinichampa (AAB)

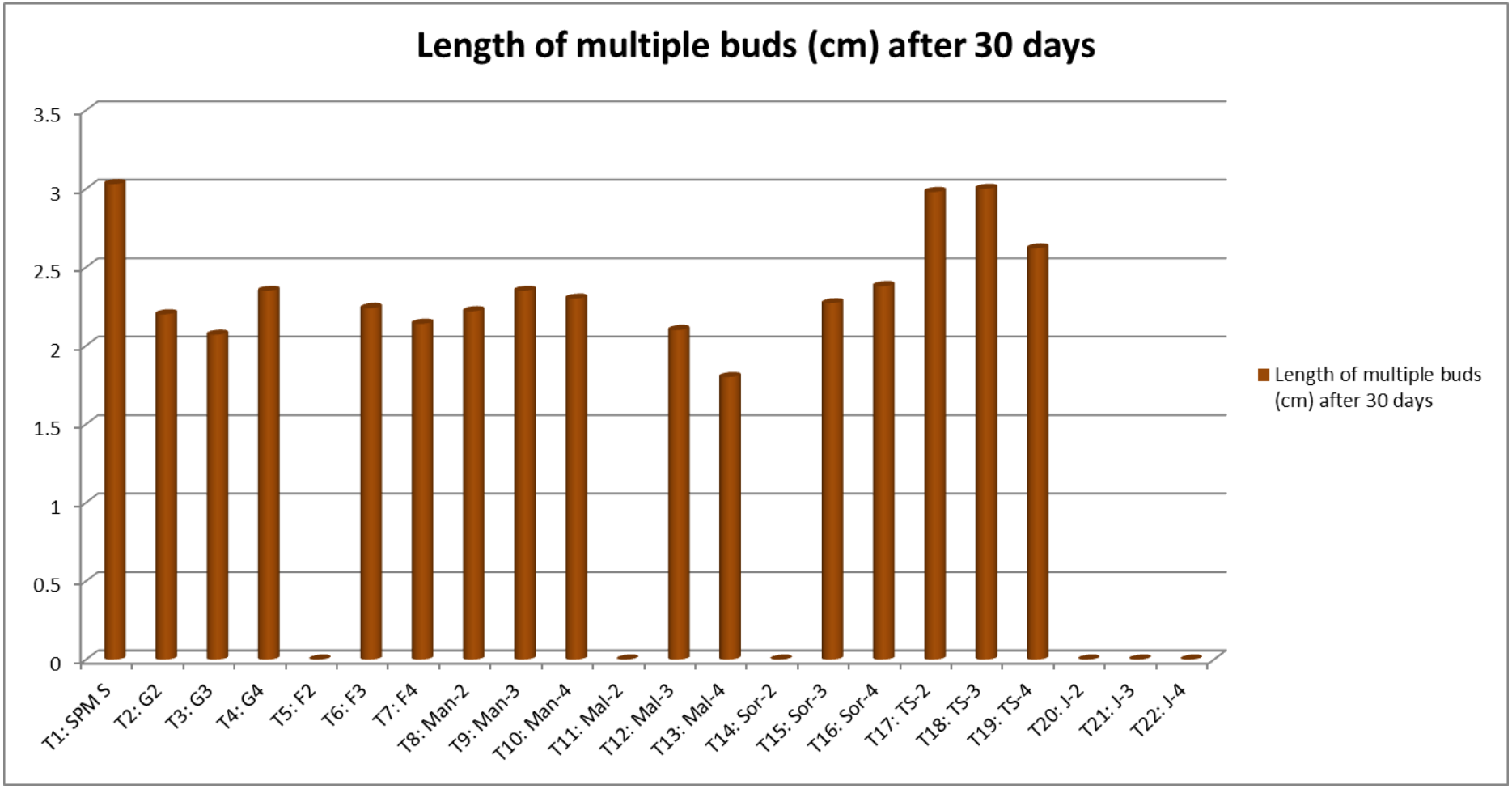
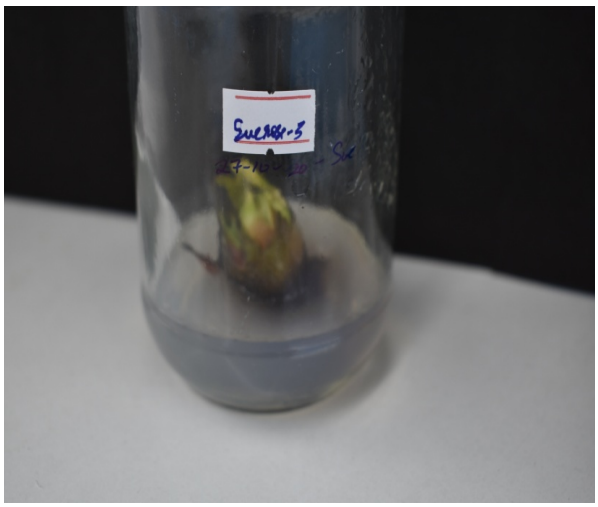
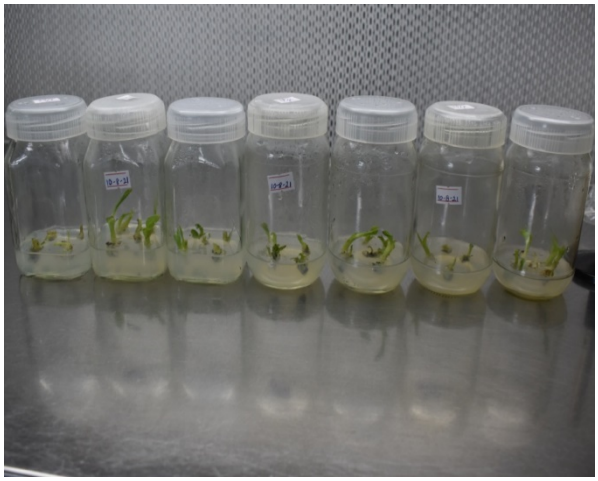


Figure 4.7 Effect of modified MS medium supplemented with various concentrations of carbon sources on length of multiple buds (cm) after 30 days of banana explants (shoot tip) cv. Chinichampa (AAB)

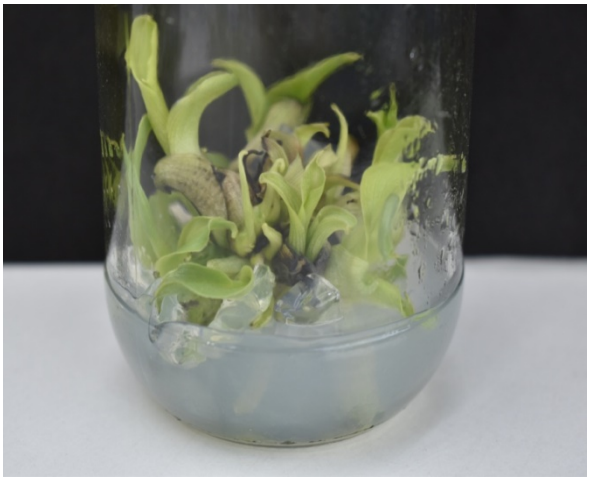


a. Swelling of shoot tip in T_1: SPM S

b. Greening of shoot tip in T_1: SPM S

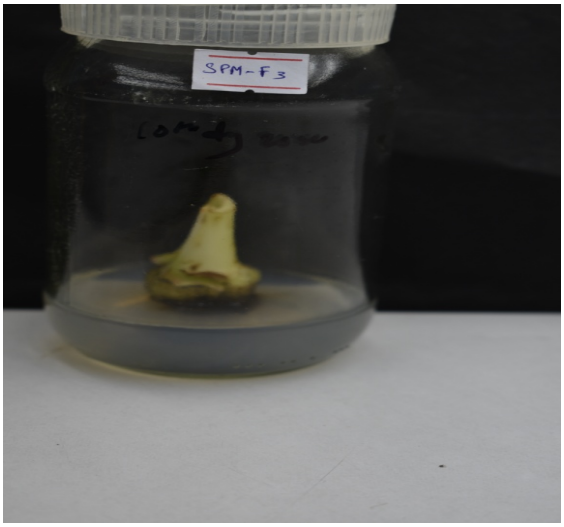


c. Multiple buds in T_1: SPM S

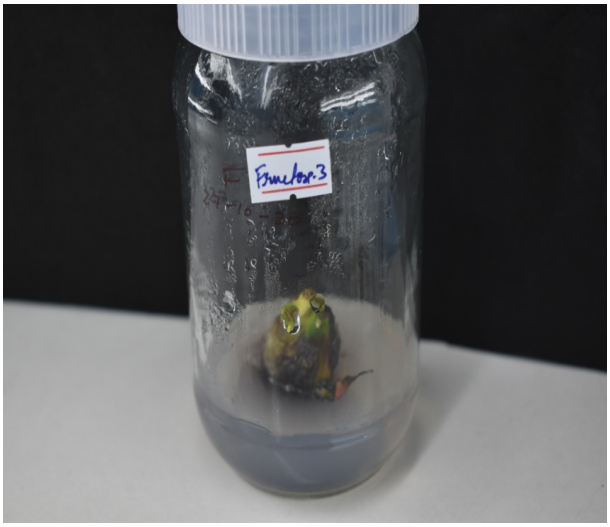


d. Elongation of multiple buds in T_1: SPM S

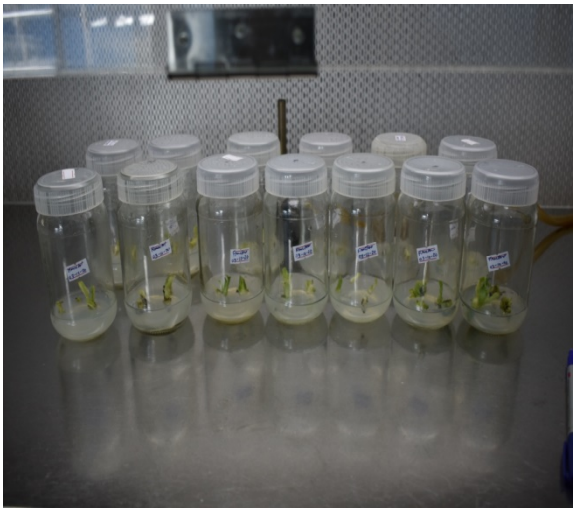
Plate 3 Effect of sucrose as carbon source in culture establishment of banana explants (shoot tip) cv. Chinichampa (AAB)



a. Initiation in T_6: SPM F3



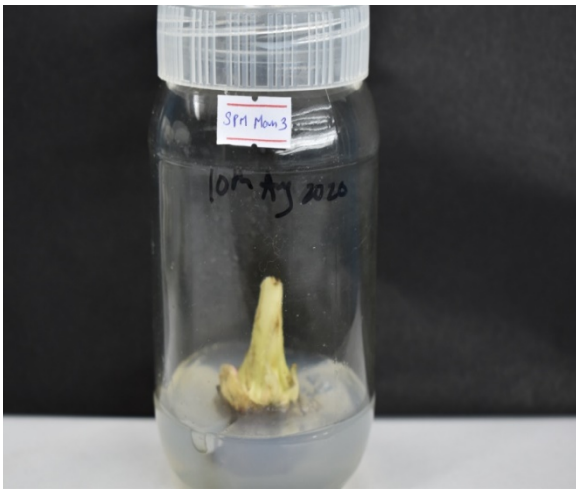
b. Greening and swelling



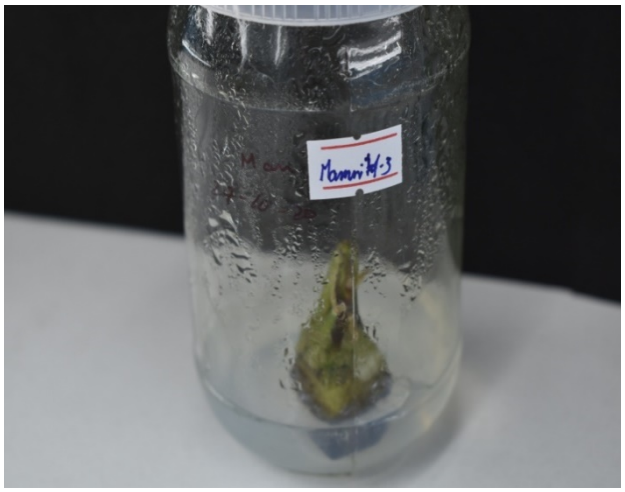
c. Multiple buds in T_6: SPM F3

d. Multiple buds in T_7: SPM F4

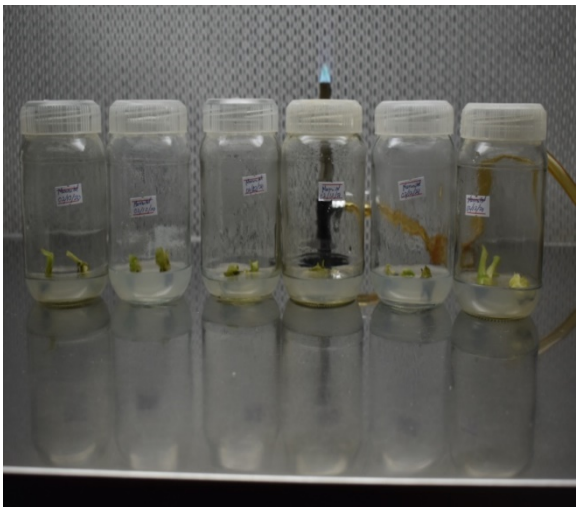
Plate 4 Effect of fructose as carbon source in culture establishment of banana explants (shoot tip) cv. Chinichampa (AAB)



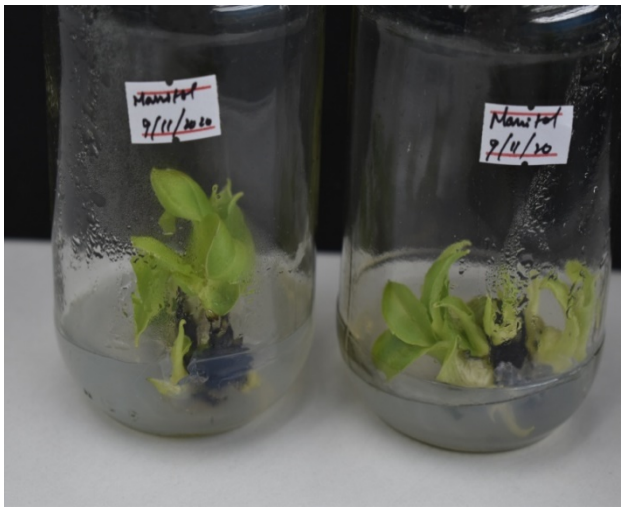
a. Initiation in T_9: SPM Man-3



b. Greening and swelling

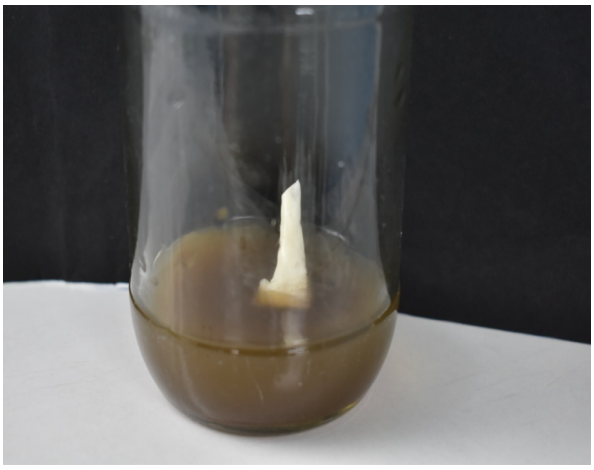


c. Multiple buds in T_{10}: SPM Man-4

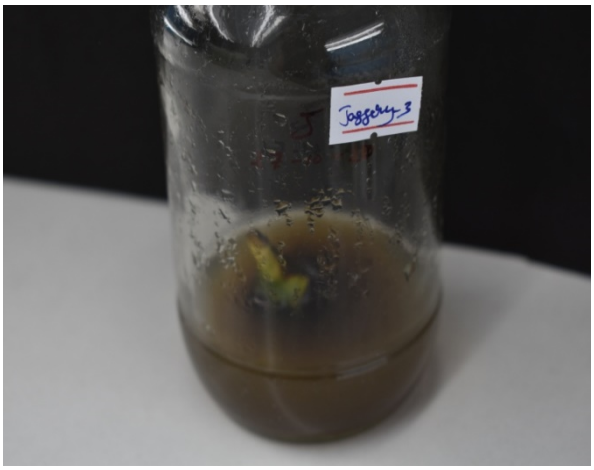


d. Elongation of multiple buds in T_{10}: SPM Man-4

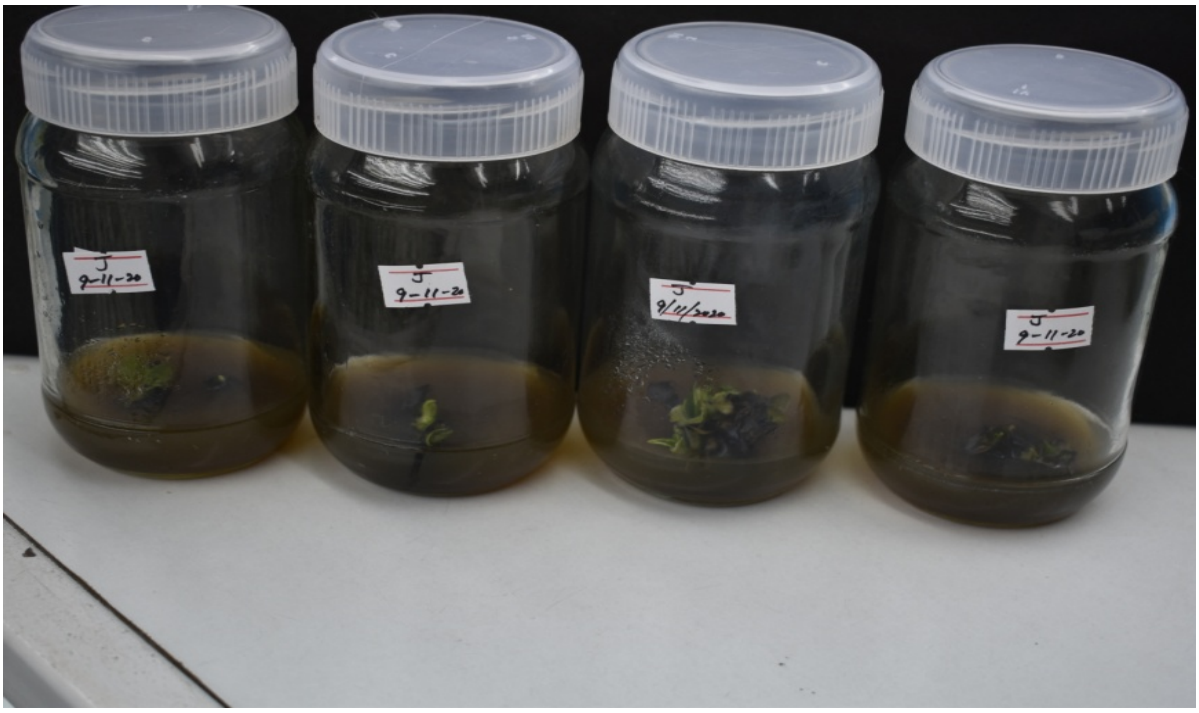
Plate 5 Effect of mannitol as carbon source in culture establishment of banana explants (shoot tip) cv. Chinichampa (AAB)



a. Initiation in T_{21}: SPM J-3

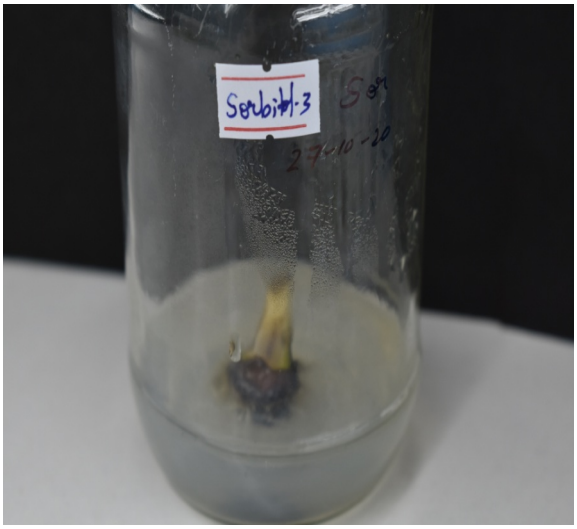


b. Greening in T_{21}: SPM J-3

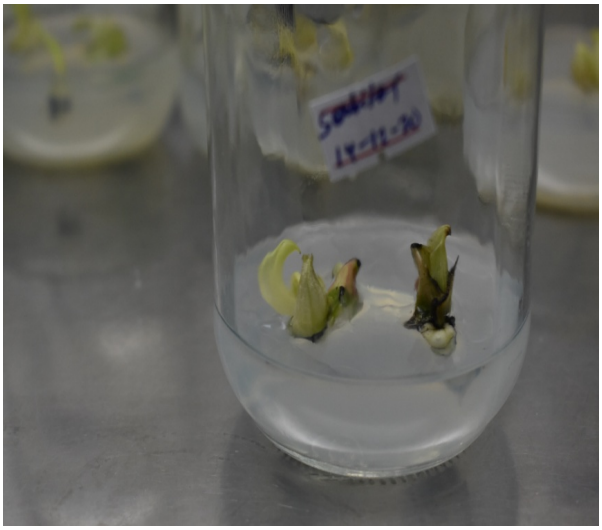


c. Culture establishment in T_{21}: SPM J-3

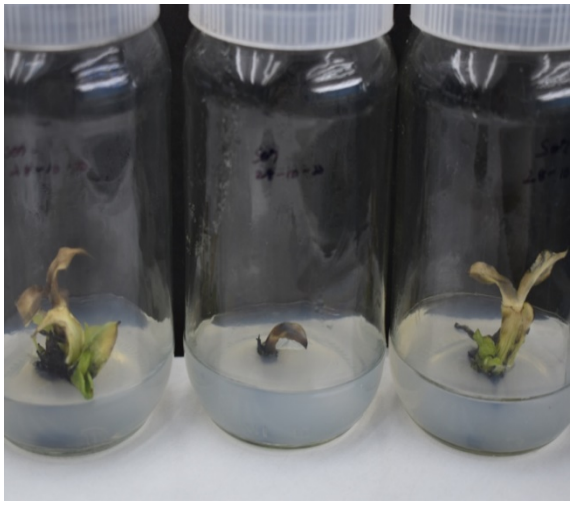
Plate 6 Effect of jaggery as carbon source in culture establishment of banana explants (shoot tip) cv. Chinichampa (AAB)



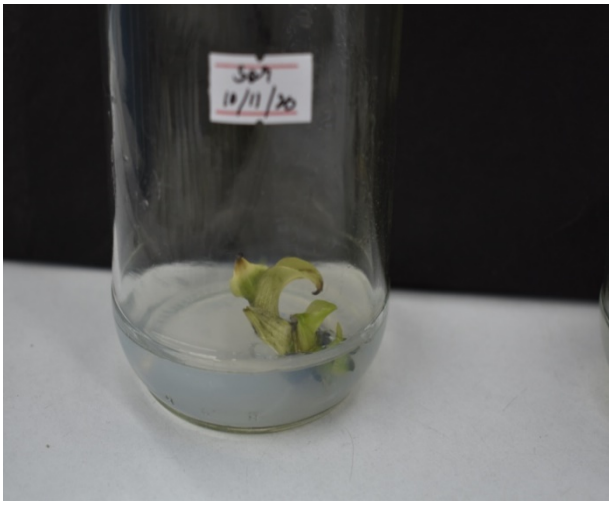
a. Initiation in T_{15}: SPM Sor-3



b. Multiple buds in T_{15}: SPM Sor-3

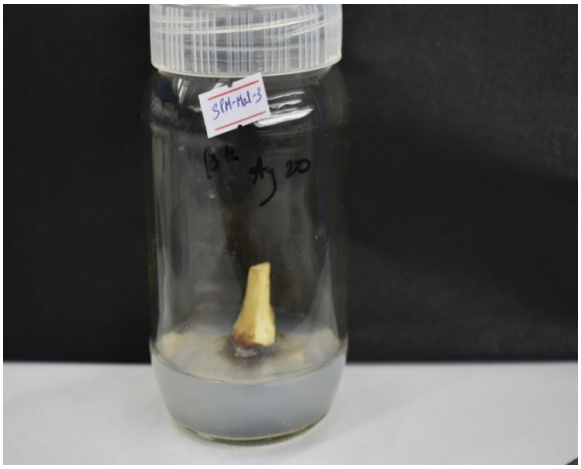


c. Multiple buds in T_{16}: SPM Sor-4

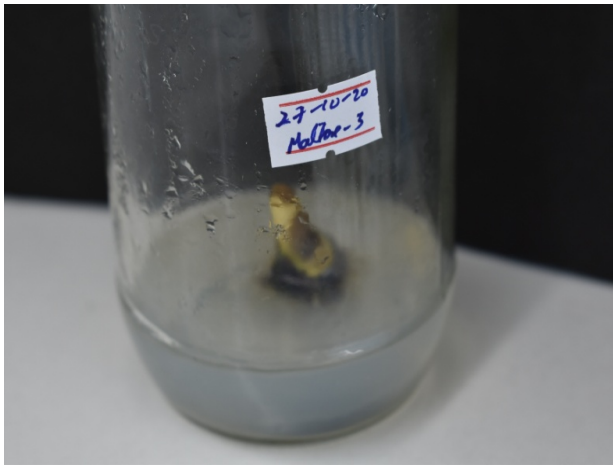


d. Elongation in T_{16}: SPM Sor-4

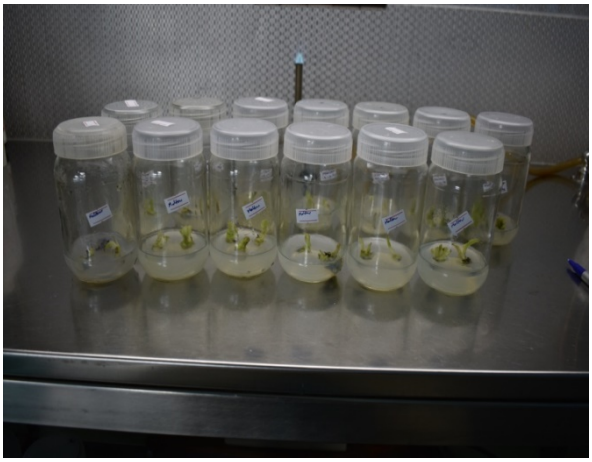
Plate 7 Effect of sorbitol as carbon source in culture establishment of banana explants (shoot tip) cv. Chinichampa (AAB)



a. Initiation in T_{12}: SPM Mal-3



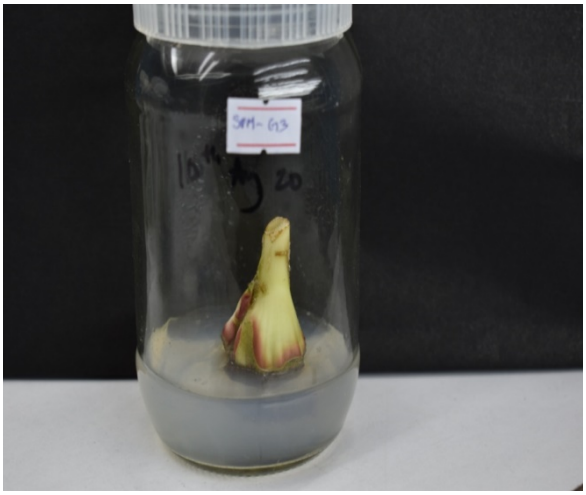
b. Greening in T_{12}: SPM Mal-3



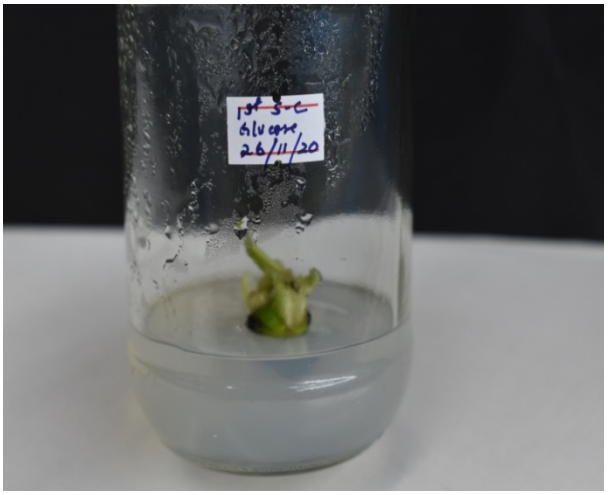
c. Multiple buds in T_{12}: SPM Mal-3

d. Elongation in T_{12}: SPM Mal-3

Plate 8 Effect of maltose as carbon source in culture establishment of banana explants (shoot tip) cv. Chinichampa (AAB)



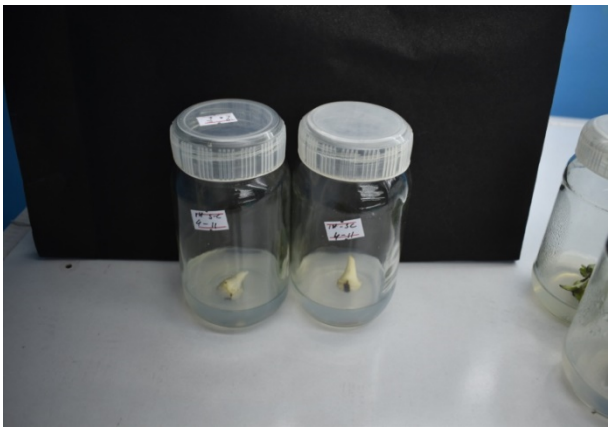
a. Swelling in T_3: SPM G-3



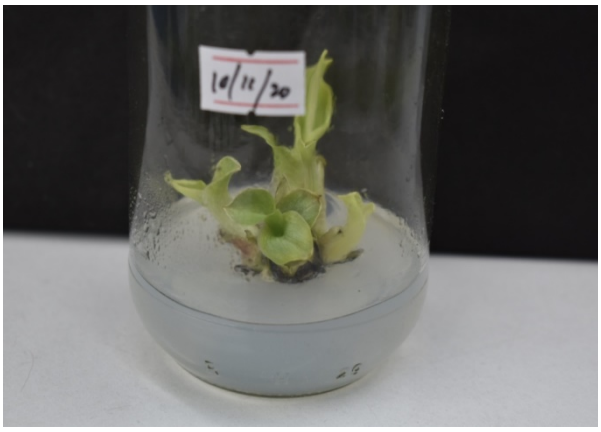
b. Greening in T_3: SPM G-3

c. Elongation in T_3: SPM G-3

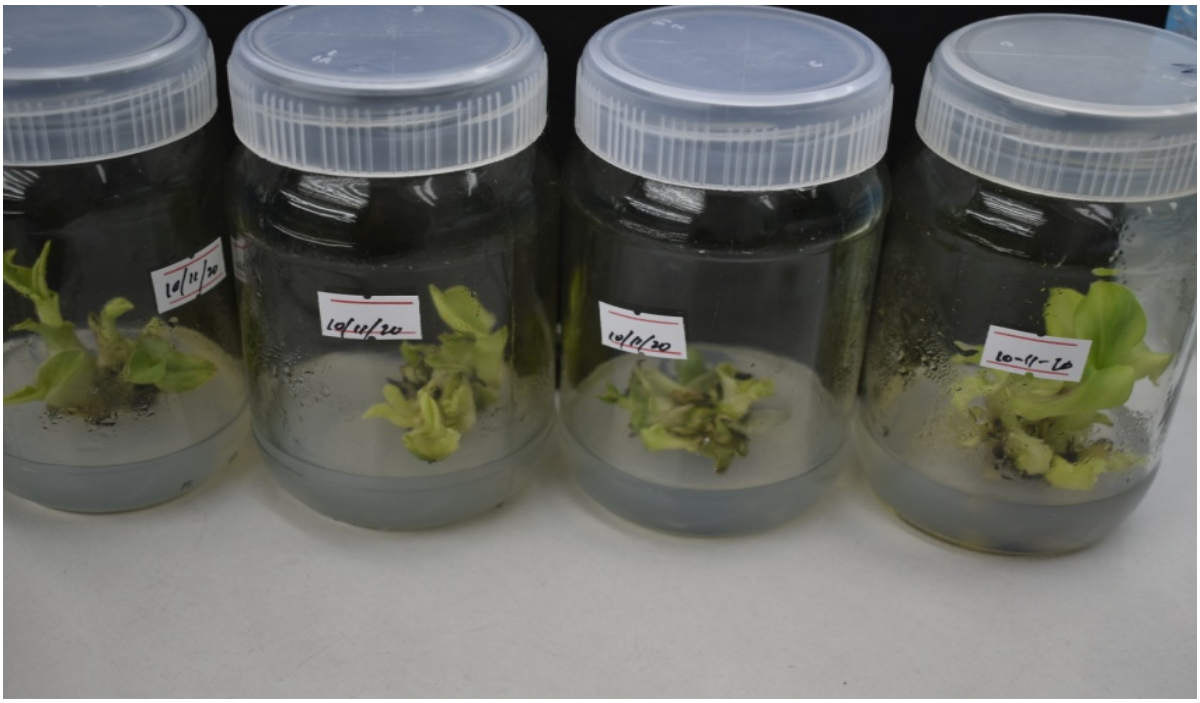
Plate 9 Effect of glucose as carbon source in culture establishment of banana explants (shoot tip) cv. Chinichampa (AAB)



a. Initiation in T_{18}: SPM TS-3



b. Multiple buds in T_{18}: SPM TS-3



c. Multiple buds in T_{19}: SPM TS-4

Plate 10 Effect of table sugar as carbon source in culture establishment of banana explants (shoot tip) cv. Chinichampa (AAB)

4.2 Experiment – 2

In this experiment, the effect of various concentrations of carbon sources were tested for *in vitro* culture establishments and shoot proliferations of banana cv. Chinichampa (AAB) through immature male floral bud (MFBs). The findings of the investigation has been properly documented and portrayed in table and graphically depicted through column chart.

4.2.1 Days to greening

Days required to greening were recorded by visually inspecting the inoculated cultures for changes in colour and the day was recorded. It was observed that the minimum days required for greening was showed in T_{18} (7.59 days) which was significantly similar with T_2 (8.00 days), T_3 (8.00 days), T_{17} (8.06 days) and T_{19} (8.25 days). The maximum number of days for greening was showed in T_{14} (13.33 days). Some treatments did not produce any results as the explants turned brown and necrotic which has also been reported in cultivar Ambun (Hernandez and Garcia, 2008). Kavitha *et al*. (2020) found that the *in vitro* response of floral buds varied from 2 to 3 weeks of culture depending on the size and physiological maturity of the floral hands. In a findings by Resmi and Nair (2007) and Darvari *et al*. (2010) the earliest greening was recorded at 16.80 days in a medium with BAP alone at 10 mg/l.

4.2.2 Days to swelling

The data regarding days taken for swelling has been shown in table 4.2 and graphically in figure 4.9. The minimum days required for swelling was recorded in T_4 (16.17 days) followed by T_1 (23.03 days) and T_{17} (23.83 days) which was statistically at par with T_{18} (24.50 days) and T_{19} (24.11 days). While T_{13} (42.17 days) required maximum days for swelling. The depicted data also revealed that T_8, T_{15}, T_{16}, T_{20}, T_{21} and T_{22} did not response to the treatments. In the process of the treatments the explants turned green and got swollen at the basal portion. The carbon source, sucrose is broken down during autoclaving

and transformed to glucose and fructose by the action of invertase enzyme (Pierik, 1987). Glucose is then utilized first and followed by fructose. Similar observation has also been reported by Hrashel *et al*. (2014) for *in vitro* propagation of immature male flowers of banana cv. Vaibalhla. Placid *et al*. (2012) reported that mannitol is not a good source of carbon for banana plantlets production under *in vitro* conditions. This might be because mannitol exerts a higher osmotic stress which causes negative impacts and strongly inhibits plant cell, tissue and organ growth.

4.2.3 Culture established (%)

Table 4.2 and figure 4.10 revealed the culture establishment percentage as recorded during the experiment. The highest culture establishment was observed in T_1 (36.67%) followed by T_{18} (23.34%) and T_6 (20.00%). Lowest percentage of culture establishment was observed in T_{14} (3.33%). Meanwhile no culture establishment was recorded in T_8, T_{15}, T_{16}, T_{20}, T_{21} and T_{22}. In Virupakshi and Sirumalai banana, culture establishment was recorded at 94.4% in medium containing BAP (5mg/l) and coconut water (15%) (Shelake *et al*., 2011).

4.2.4 Days required for multiple buds initiation

The data for days required for multiple buds initiation has been presented in table 4.2 and graphically in figure 4.11. From the data the minimum days required for multiple buds initiation was in T_1 (44.11) which was statistically at par with T_{17} (45.83), T_{18} (44.67) and T_{19} (46.50) followed by T_2 (51.33). Statistical analysis also revealed that the days required for multiple buds initiation does not vary significantly for most of the treatments. In Rasthali (AAB) banana, Kavitha *et al*. (2020) revealed that multiple buds initiation begins within 4 weeks and continue till the 8^{th} week depending on the treatments. It further observed that early initiation of multiple buds was observed in medium with BAP alone at 10 mg/l. followed by TDZ alone at 0.4

mg/l. Similar findings were also reported by Resmi and Nair (2007) and Darvari *et al*. (2010).

4.2.5 Culture showing multiple buds (%)

The culture showing multiple buds were recorded and expressed in percent as shown in table 4.2 and figure 4.12. The maximum percentage of multiple buds was found in T_1 (20.00%) followed by T_{18} (16.67%). Experimental results also revealed that there was no multiple buds initiation in treatments T_8, T_{14}, T_{15}, T_{16}, T_{20}, T_{21} and T_{22}. In many species maltose also stimulates callus, probably when it is hydrolyzed into glucose it increases regeneration better than sucrose or glucose, as observed in *Oryza sativa* (Kumrai *et al*., 2001) and *Glycine max* (Sairam *et al*., 2003). Sorbitol (sugar alcohol) resulted in very little or no callus induction on both the leaf and immature inflorescences of *Miscanthus* (Peterson *et al*., 1999). Similar results were observed in this experiment. It is likely that sorbitol was not properly metabolized by the *Musa spp*. tissues, which in turn brought about carbon starvation, without killing the inoculated explants.

4.2.6 Number of multiple buds produced per explant

The observation recorded for multiple buds produced per explant has been recorded in table 4.2 and graphically depicted in figure 4.13. Highest number of multiple buds was recorded in T_{18} (8.33) which was statistically at par with T_1 (8.28) followed by T_{19} (7.17) and T_3 (6.67), T_4 (6.17), T_6 (6.00) and T_{17} (6.33) which were all at par. Low multiple buds production was observed in T_{11} (3.50). Several studies in banana reported that shoot multiplication depended on the genotype of cultivars (Resmi and Nair, 2007; Israeli *et al.,* 1995 and Mendes *et al*., 1999). *In vitro* shoot proliferation using immature male flower buds of Poovan (AAB) Nair *et al*. (2018) obtained maximum bud (16.00) development in medium containing 6 mg/l BAP. However, Smitha *et al*. (2014) reported the influence of both genotypes and explants size on the rate of shoot multiplication.

4.2.7 Length of multiple buds (cm) after 30 days

Length of all the multiple buds produced per explant was measured by using iron graduated scale starting from base of collar region to the top newly emerged leaf; their mean was worked out in centimeters and the data has been shown in table 4.2 and figure 4.14. Maximum length of multiple buds after 30 days was recorded in T_1 (3.01 cm) which was statistically at par with T_{17} (2.75 cm) and T_{18} (2.96 cm) followed by T_3 (2.17 cm) and which was at par with T_5 (2.12 cm) and T_6 (2.12 cm). Shelake *et al*. (2011) revealed that shoot elongation was maximum in medium containing BAP (5 mg/l) and GA (1.5 mg/l) in Virupakshi and Sirumalai banana. Similar findings were also reported in Poovan (AAB) by Nair *et al*. (2018).

Table 4.2 Effect of modified MS medium supplemented with various concentrations of carbon sources on *in vitro* culture establishment and shoot proliferation of banana explants (male floral bud) cv. Chinichampa (AAB)

Treatments	Days to greening	Days to swelling	Culture established (%)	Days required for multiple buds initiation	Culture showing multiple buds (%)	Number of multiple buds produced per explant	Length of multiple buds (cm) after 30 days
T_1: SPM S	8.73^{cd}	23.03^{f}	36.67	44.11^{c}	20.00	8.28^{a}	3.01^{a}
T_2: SPM G2	8.00^{d}	27.00^{de}	10.00	51.33^{bc}	6.67	5.67^{cd}	1.31^{e}
T_3: SPM G3	8.00^{d}	26.33^{de}	13.34	53.67^{ab}	6.67	6.67^{bc}	2.17^{bc}
T_4: SPM G4	8.83^{cd}	16.17^{g}	13.34	54.83^{ab}	10.00	6.17^{bc}	2.02^{cd}
T_5: SPM F2	9.17^{cd}	29.33^{cd}	6.67	56.67^{ab}	3.34	4.50^{de}	2.12^{bc}
T_6: SPM F3	9.89^{cd}	28.22^{de}	20.00	57.67^{ab}	10.00	6.00^{bc}	2.12^{bc}
T_7: SPM F4	8.67^{d}	29.67^{de}	13.34	58.00^{ab}	6.67	5.67^{cd}	1.74^{cd}
T_8: SPM Man-2	11.00^{bc}	0.00^{h}	0.00	0.00^{e}	0.00	0.00^{f}	0.00^{f}
T_9: SPM Man-3	11.83^{ab}	28.67^{de}	10.00	53.67^{ab}	6.67	5.00^{de}	2.16^{bc}
T_{10}: SPM Man-4	13.17^{ab}	28.67^{de}	6.67	58.33^{ab}	3.34	5.50^{cd}	2.06^{c}
T_{11}: SPM Mal-2	12.00^{ab}	35.00^{bc}	6.67	59.33^{ab}	3.34	3.50^{e}	1.87^{cd}
T_{12}: SPM Mal-3	12.17^{ab}	32.33^{bc}	6.67	61.33^{ab}	3.34	4.67^{de}	1.85^{cd}
T_{13}: SPM Mal-4	13.00^{ab}	35.83^{b}	10.00	66.00^{a}	6.67	4.00^{e}	1.50^{de}
T_{14}: SPM Sor-2	13.33^{a}	42.17^{a}	3.33	0.00^{e}	0.00	0.00^{f}	0.00^{f}
T_{15}: SPM Sor-3	0.00^{e}	0.00^{h}	0.00	0.00^{e}	0.00	0.00^{f}	0.00^{f}
T_{16}: SPM Sor-4	0.00^{e}	0.00^{h}	0.00	0.00^{e}	0.00	0.00^{f}	0.00^{f}
T_{17}: SPM TS-2	8.06^{d}	23.83^{ef}	13.34	45.83^{c}	10.00	6.33^{bc}	2.75^{a}
T_{18}: SPM TS-3	7.95^{d}	24.50^{ef}	23.34	44.67^{c}	16.67	8.33^{a}	2.96^{a}
T_{19}: SPM TS-4	8.25^{d}	24.11^{ef}	16.67	46.50^{c}	10.00	7.17^{b}	2.65^{ab}
T_{20}: SPM J-2	0.00^{f}	0.00^{h}	0.00	0.00^{e}	0.00	0.00^{f}	0.00^{f}
T_{21}: SPM J-3	0.00^{f}	0.00^{h}	0.00	0.00^{e}	0.00	0.00^{f}	0.00^{f}
T_{22}: SPM J-4	0.00^{f}	0.00^{h}	0.00	0.00^{e}	0.00	0.00^{f}	0.00^{f}
SEm±	*0.71*	*1.88*	-	*3.75*	-	*0.38*	*0.17*
CD (P=0.05)	*2.03*	*5.35*	-	*10.61*	-	*1.08*	*0.49*

*Means with different superscript letters within column are significantly different from each other by DMRT (*p=0.05*)

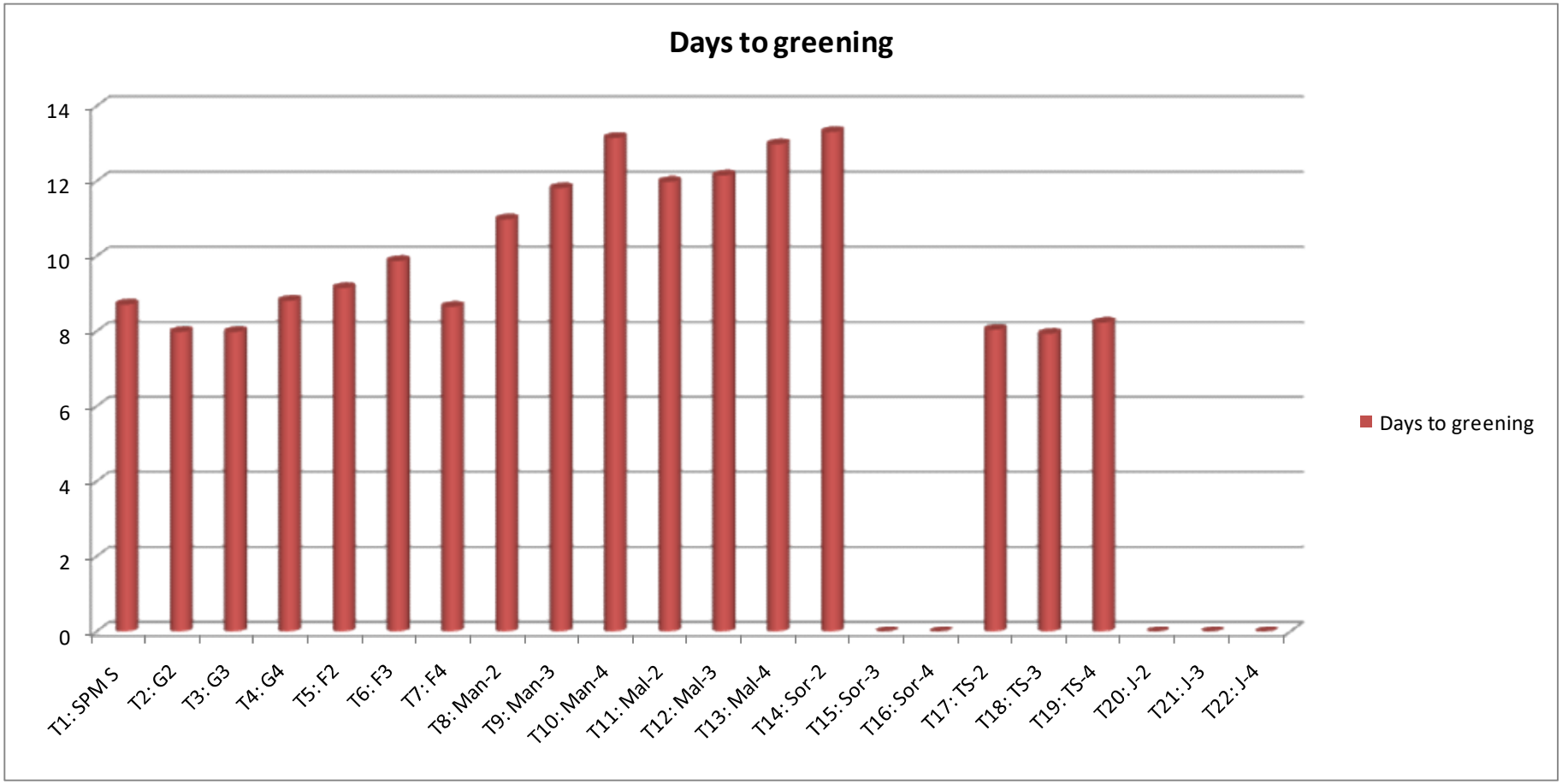


Figure 4.8 Effect of modified MS medium supplemented with various concentrations of carbon sources on days to greening of banana explants (male floral buds) cv. Chinichampa (AAB)

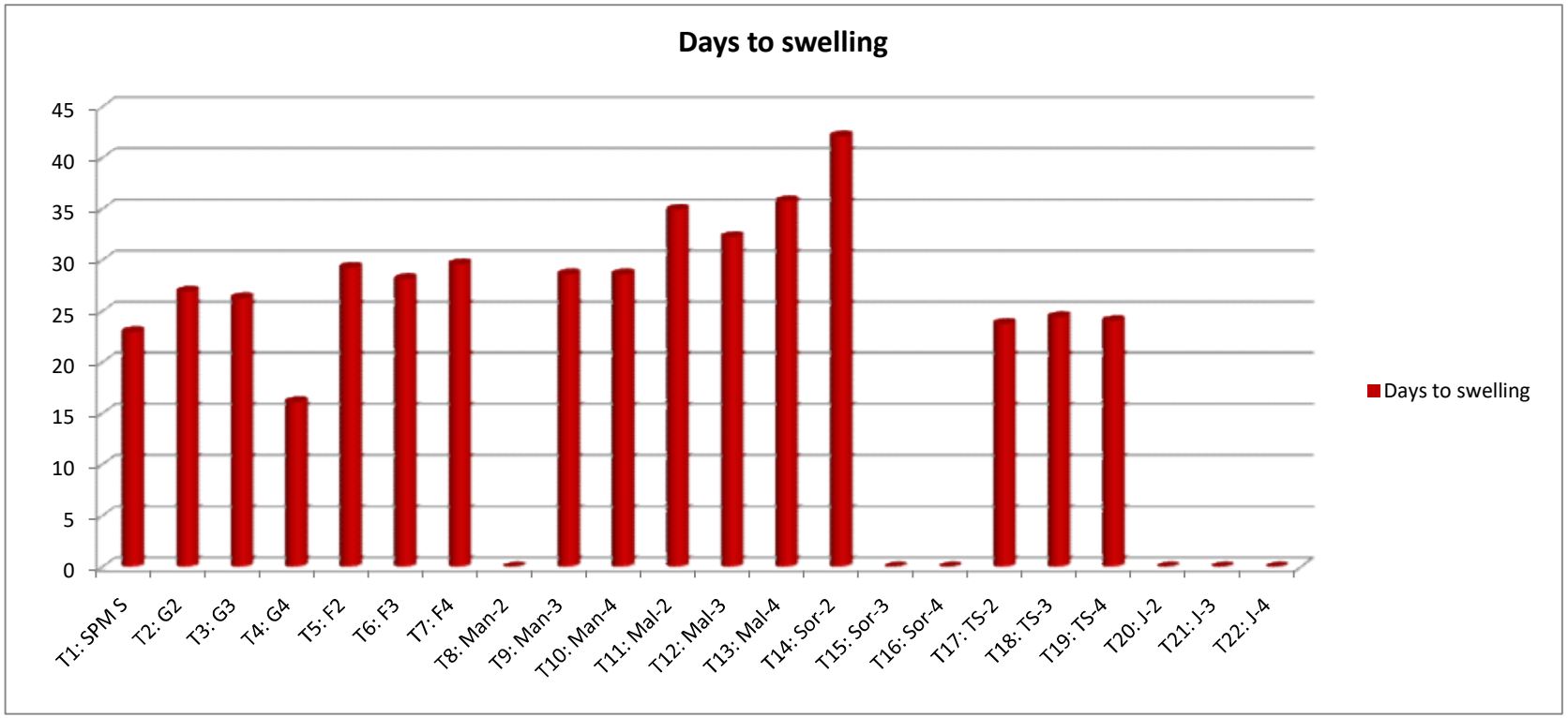


Figure 4.9 Effect of modified MS medium supplemented with various concentrations of carbon sources on days to swelling of banana explants (male floral buds) cv. Chinichampa (AAB)

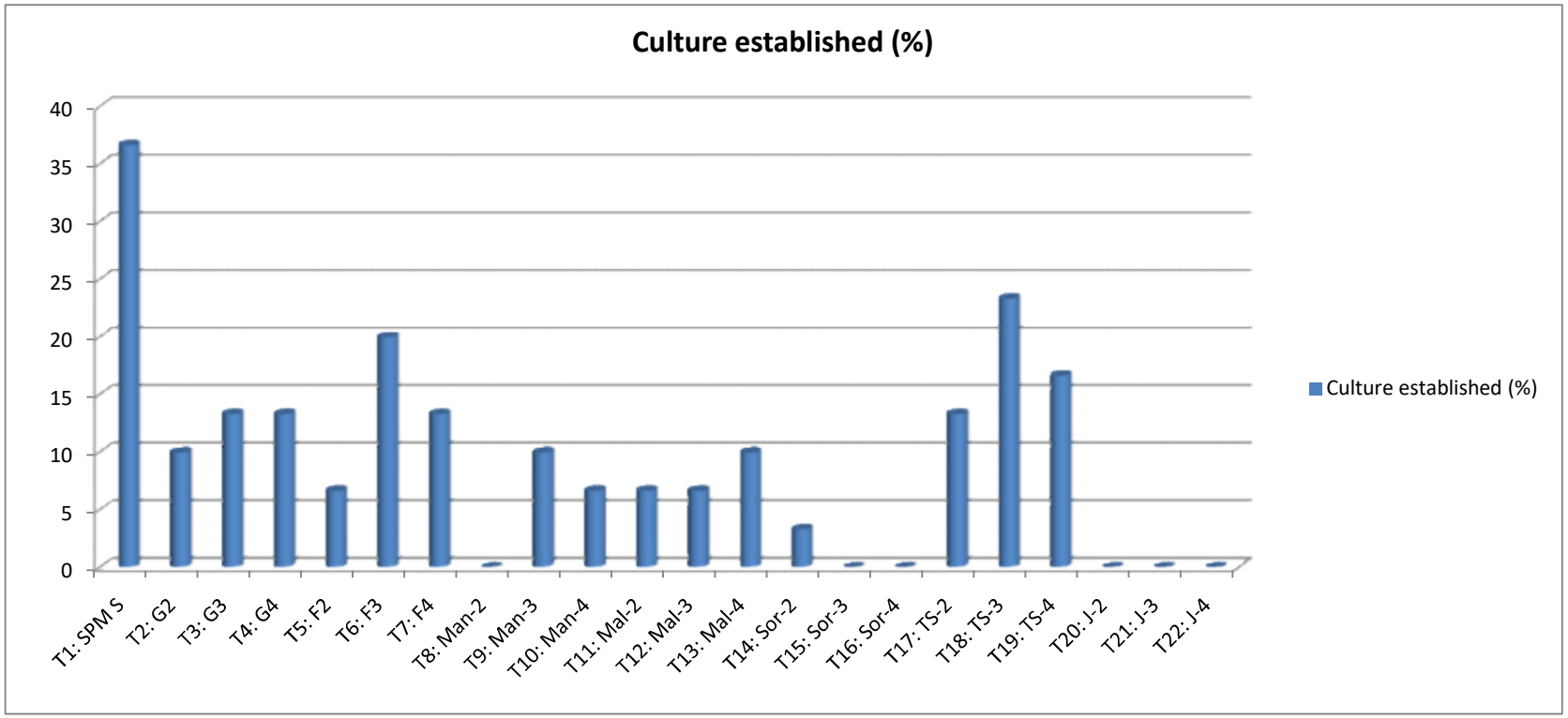


Figure 4.10 Effect of modified MS medium supplemented with various concentrations of carbon sources on culture establishment of banana explants (male floral buds) cv. Chinichampa (AAB)

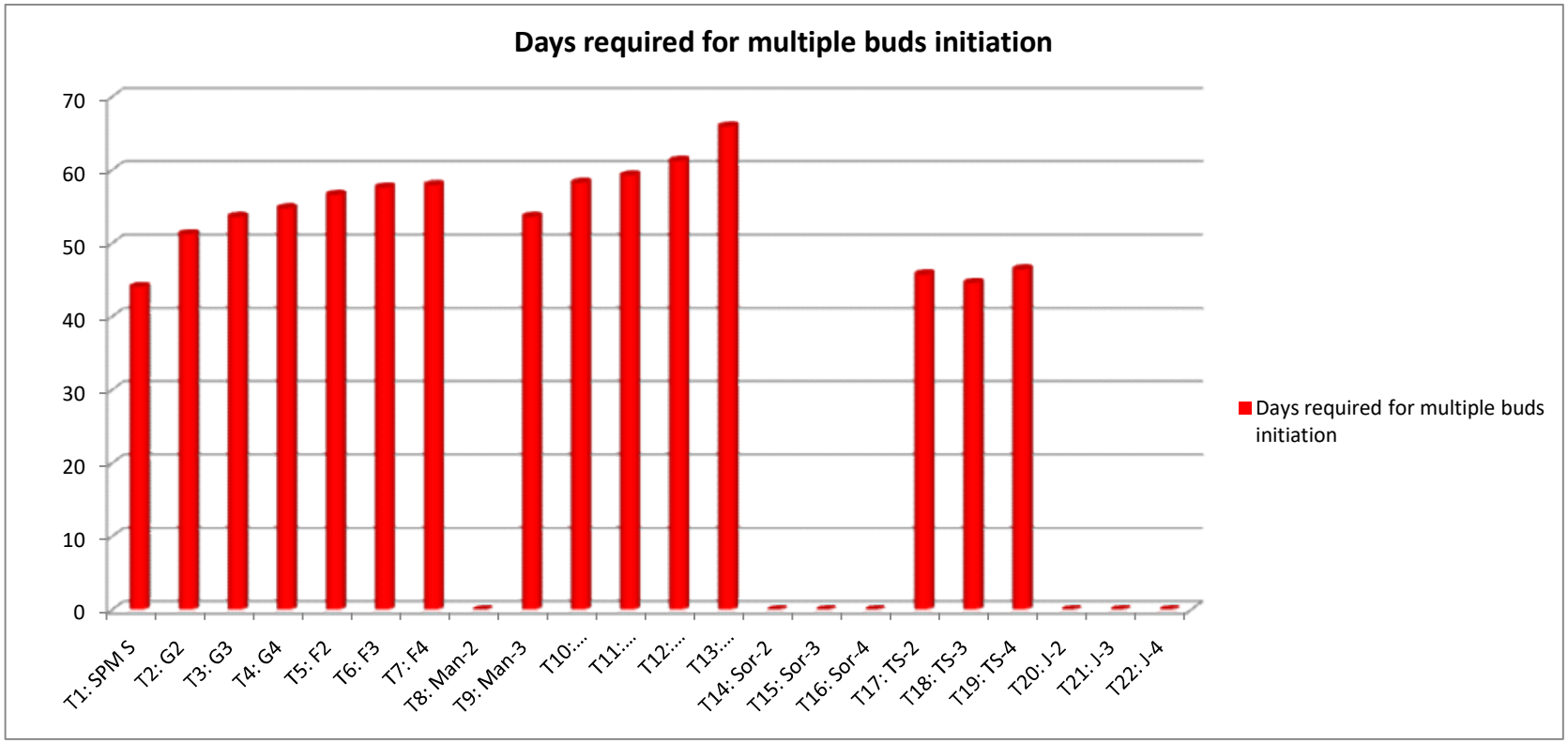


Figure 4.11 Effect of modified MS medium supplemented with various concentrations of carbon sources on days required for multiple buds initiation of banana explants (male floral buds) cv. Chinichampa (AAB)

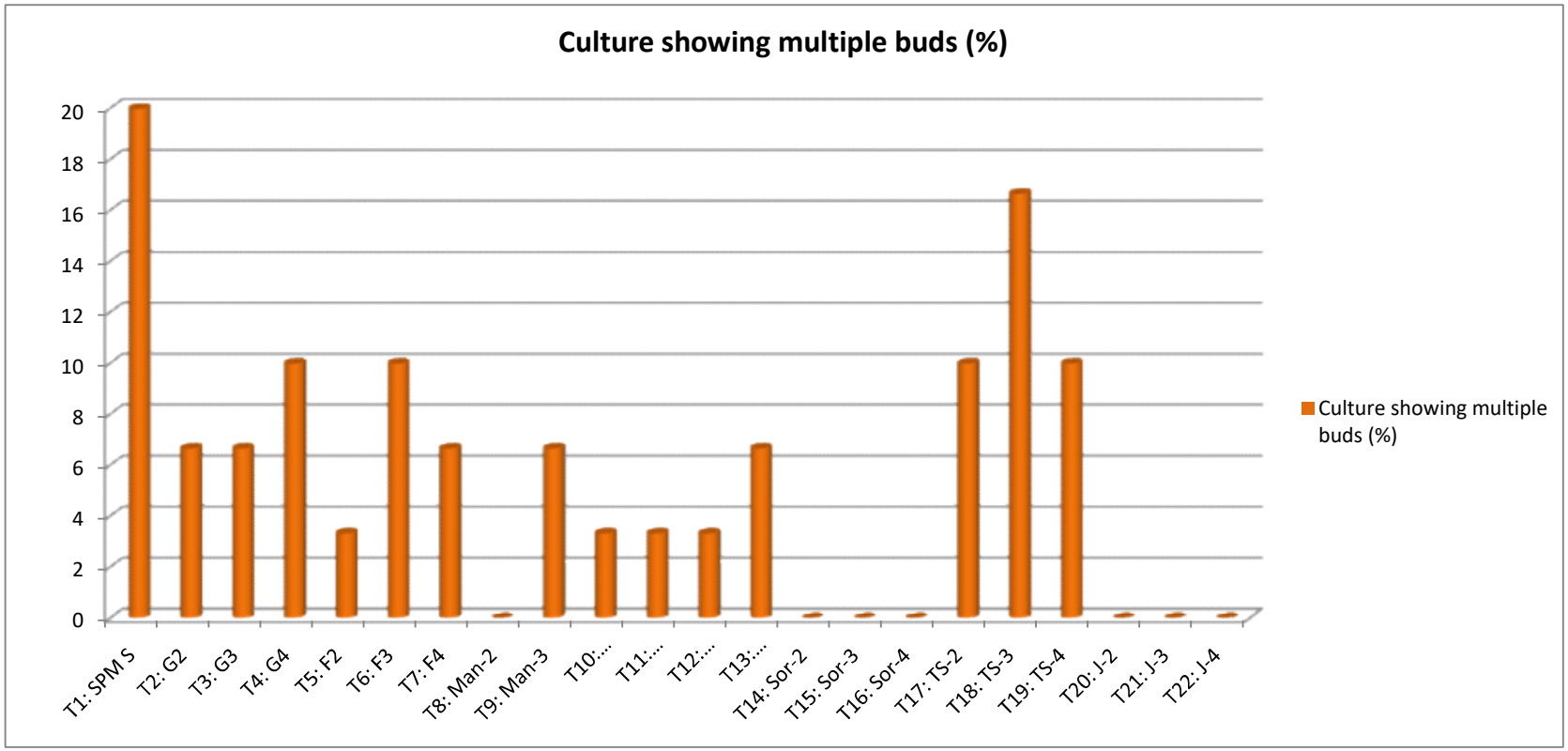


Figure 4.12 Effect of modified MS medium supplemented with various concentrations of carbon sources on culture showing multiple buds of banana explants (male floral buds) cv. Chinichampa (AAB)

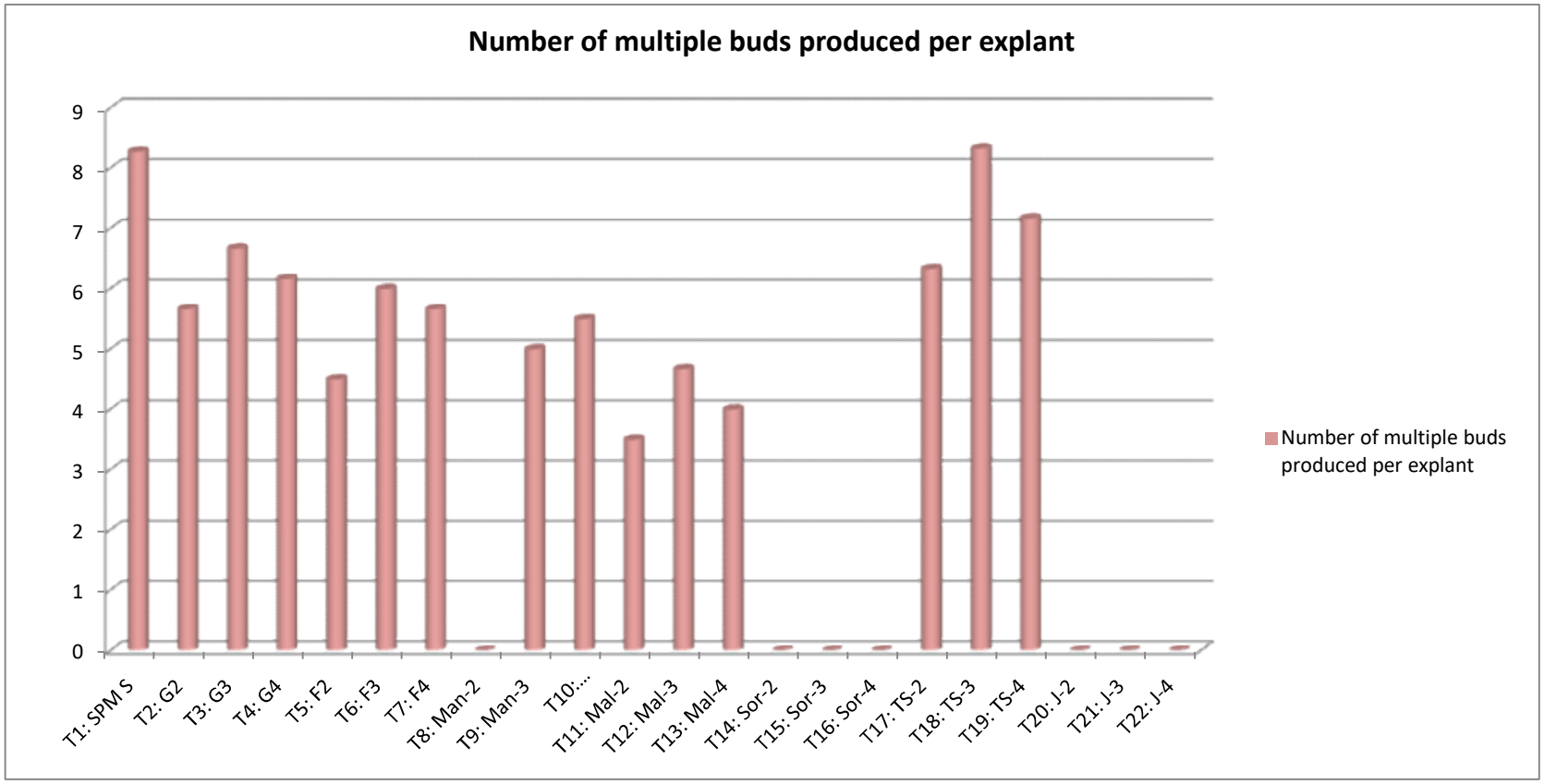


Figure 4.13 Effect of modified MS medium supplemented with various concentrations of carbon sources on number of multiple buds produced per explants of banana explants (male floral buds) cv. Chinichampa (AAB)

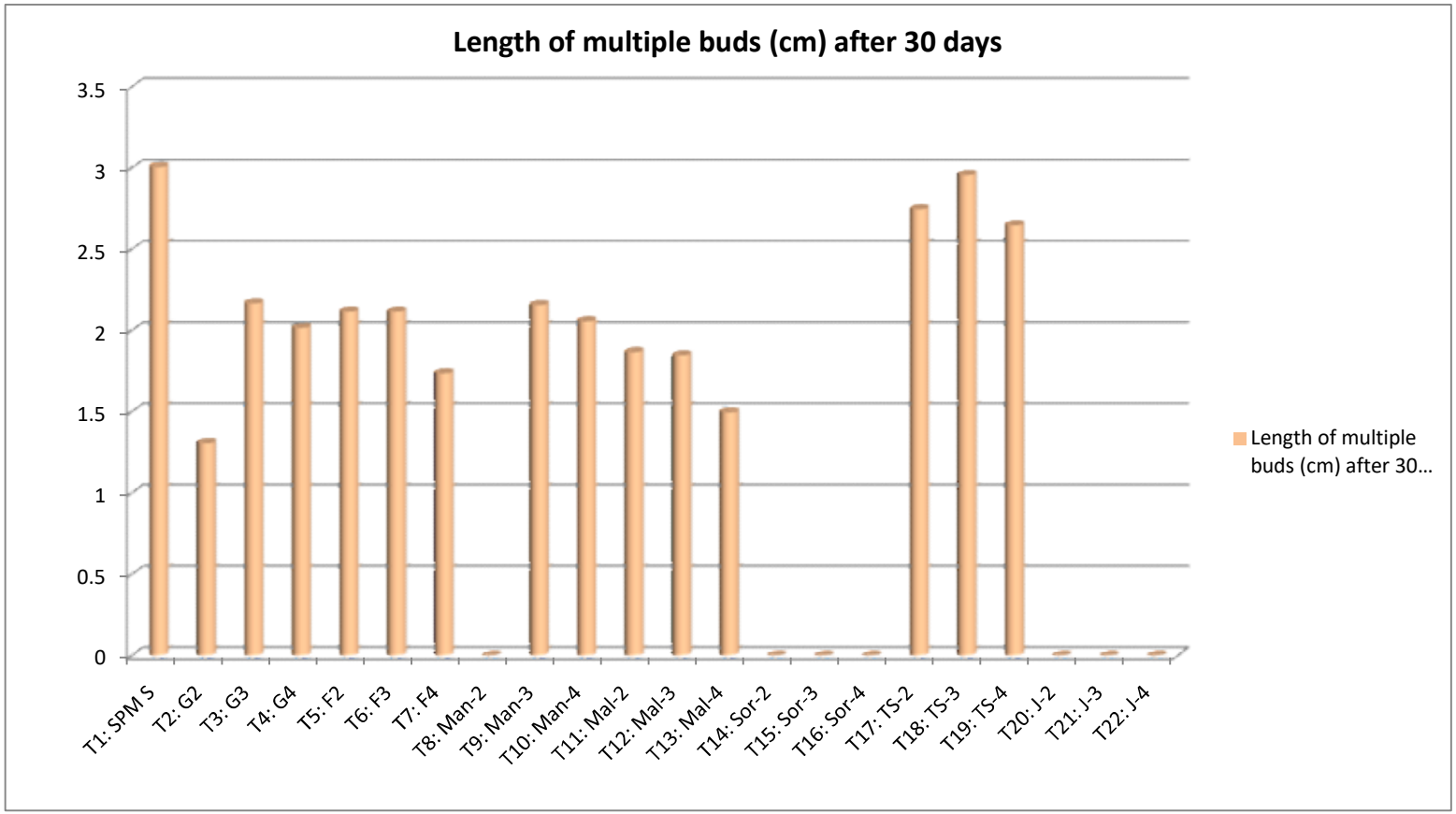
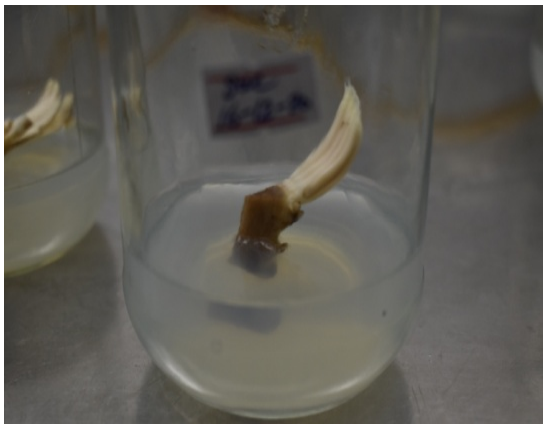
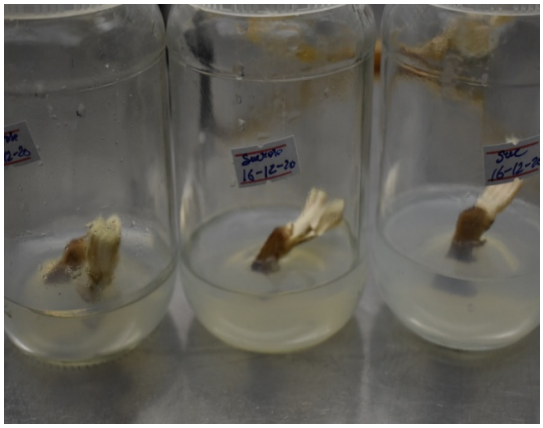


Figure 4.14 Effect of modified MS medium supplemented with various concentrations of carbon sources on length of multiple buds (cm) after 30 days of banana explants (male floral buds) cv. Chinichampa (AAB)

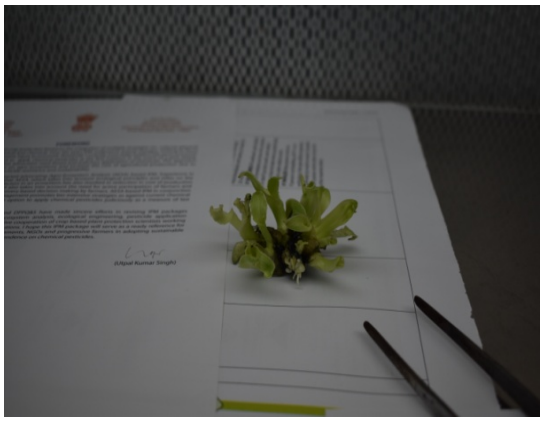


Initiation in T_1: SPM S

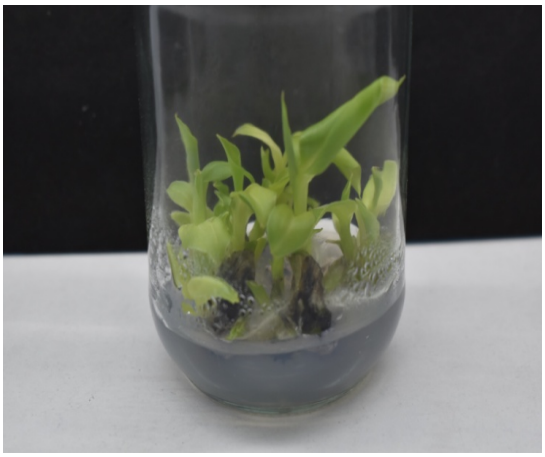


Greening in T_1: SPM S

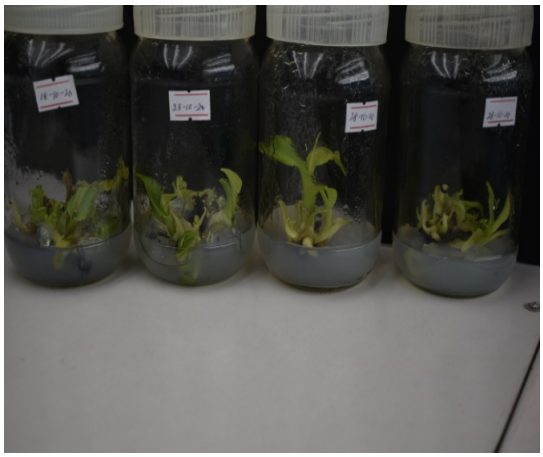
Multiple buds in T_1: SPM S



Subculturing



Elongation of multiple shoots

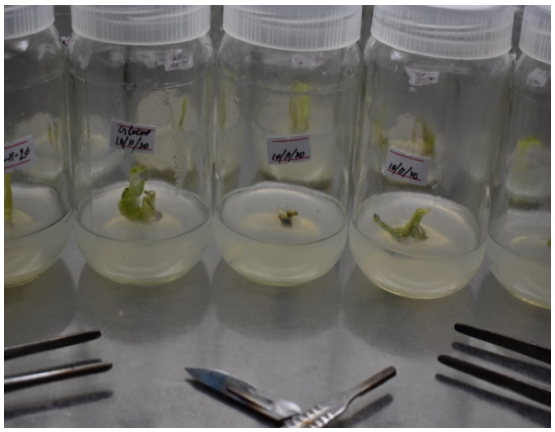


Multiple shoots regeneration

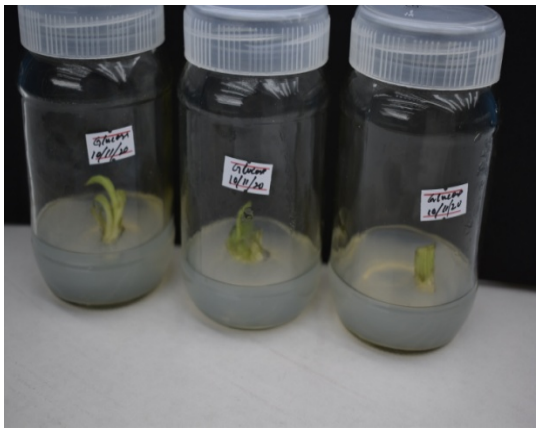
Plate 11 Effect of Sucrose in culture establishment of banana explants (MFBs) cv Chinichampa (AAB)

Initiation in T_3 SPM G-3

Initiation in T_4 SPM G-4



Subculturing in T_3 SPM G-3

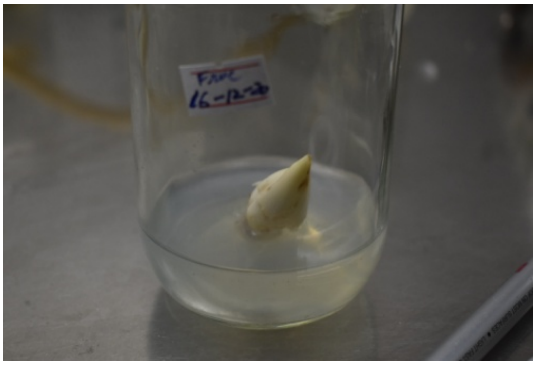


Subculturing in T_4 SPM G-4

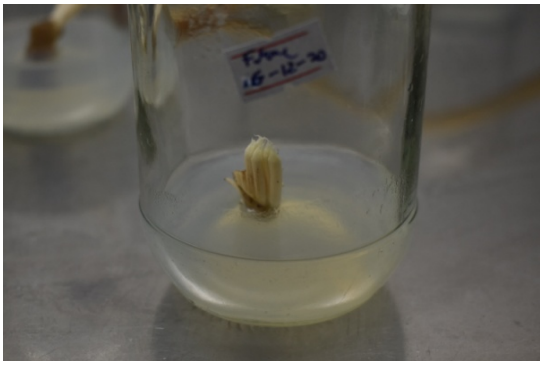
Multiple buds in T_3 SPM G-3

Elongation in T_3 SPM G-3

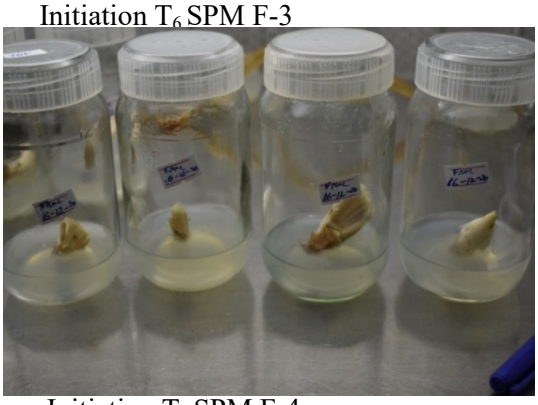
Plate 12 Effect of Glucose in culture establishment of banana explants (MFBs) cv Chinichampa (AAB)



Initiation T_6 SPM F-3

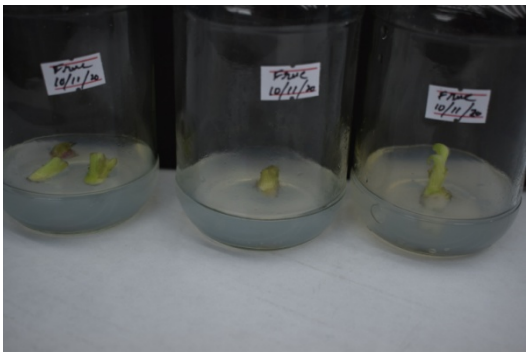


Initiation T_5 SPM F-2



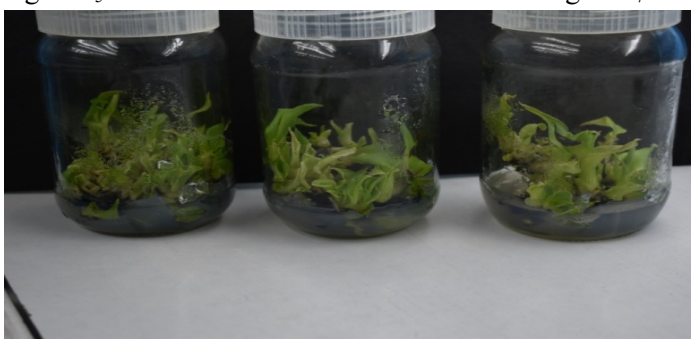
Initiation T_7 SPM F-4

Subculturing in T_6 SPM F-3



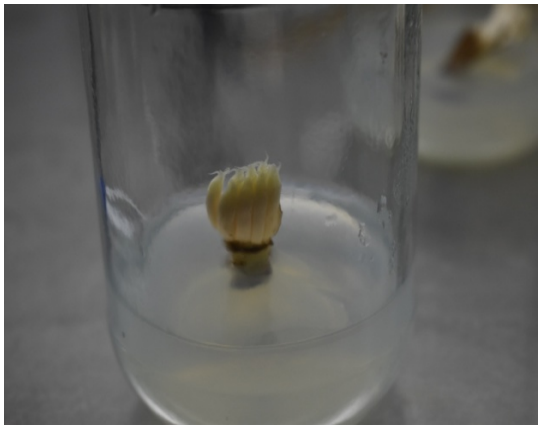
Subculturing in T_5 SPM F-2

Subculturing in T_7 SPM F-4

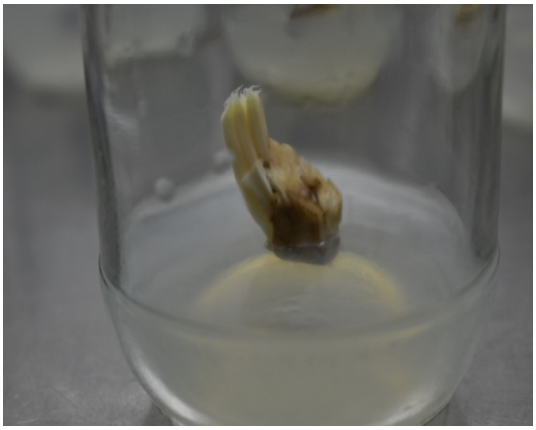


Elongation in T_6 SPM F-3

Plate 13 Effect of Fructose in culture establishment of banana explants (MFBs) cv Chinichampa (AAB)

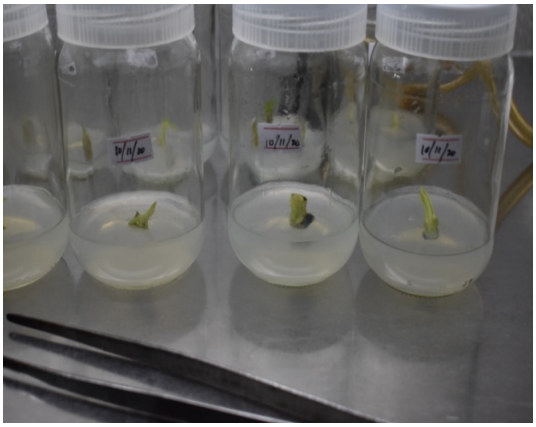


Initiation in T_9 SPM Man-3



Initiation in T_{10} SPM Man-4

Greening in T_9 SPM Man-3

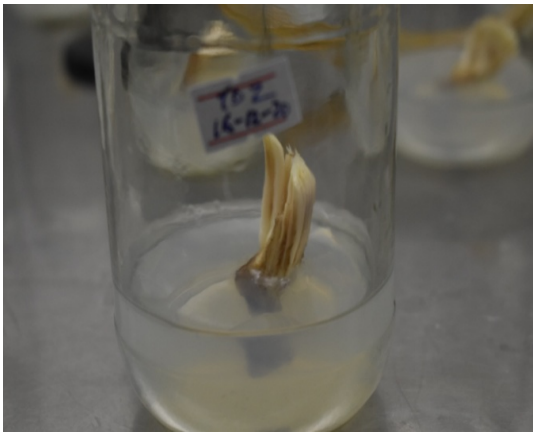


Greening in T_{10} SPM Man-4

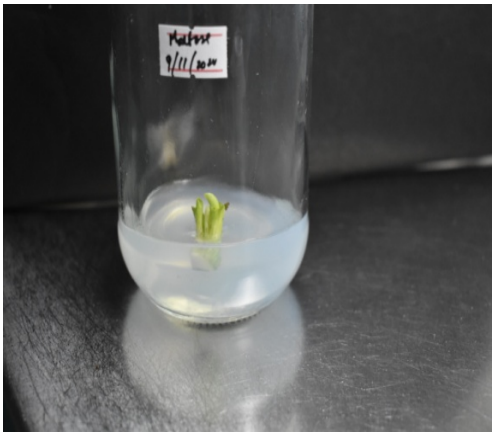
Subculturing in T_9 SPM Man-3

Elongation in T_9 SPM Man-3

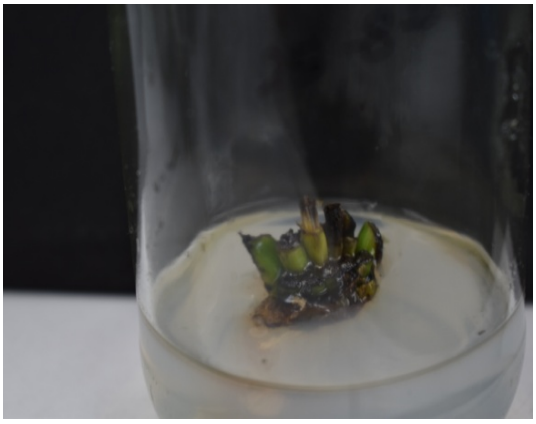
Plate 14 Effect of Mannitol in culture establishment of banana explants (MFBs) cv Chinichampa (AAB)



Initiation in T_{11} SPM Mal-2

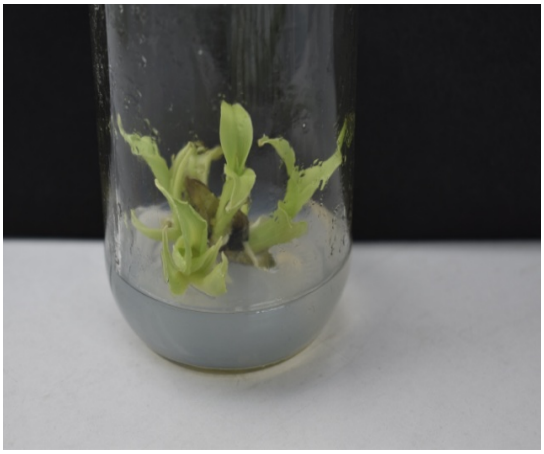


Greening in T_{11} SPM Mal-2

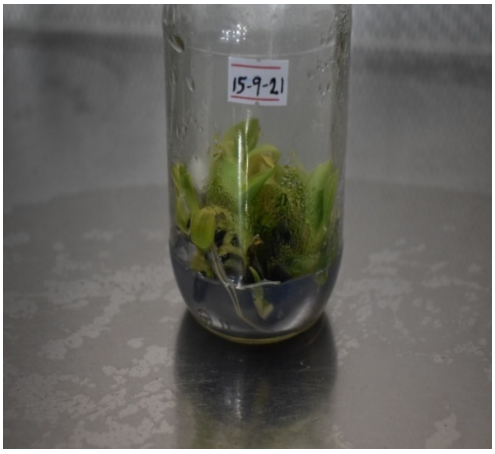


Greening in T_{12} SPM Mal-3

Subculturing in T_{11} SPM Mal-2

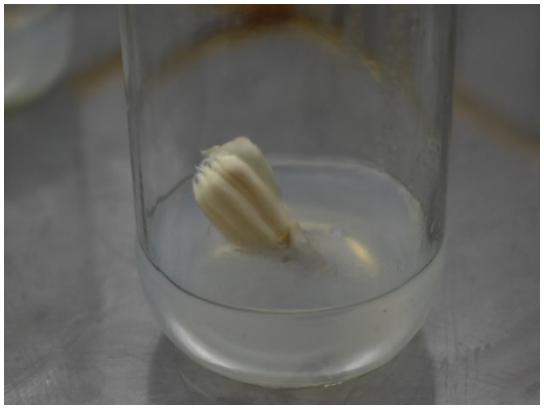


Elongation in T_{11} SPM Mal-2

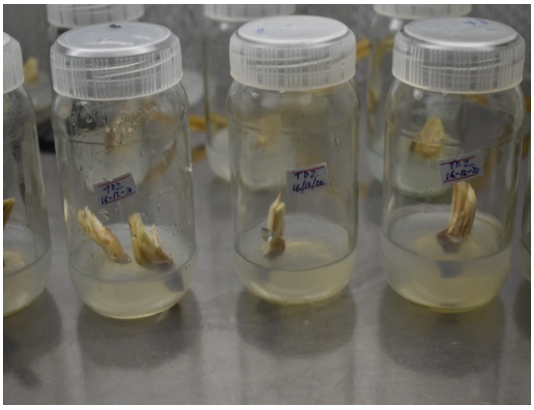


Elongation in T_{13} SPM Mal-4

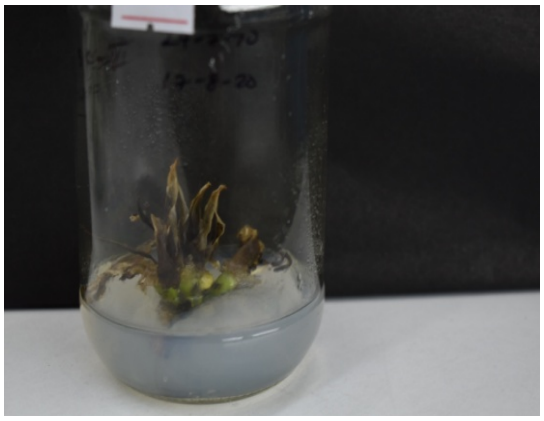
Plate 15 Effect of Maltose in culture establishment of banana explants (MFBs) cv Chinichampa (AAB)



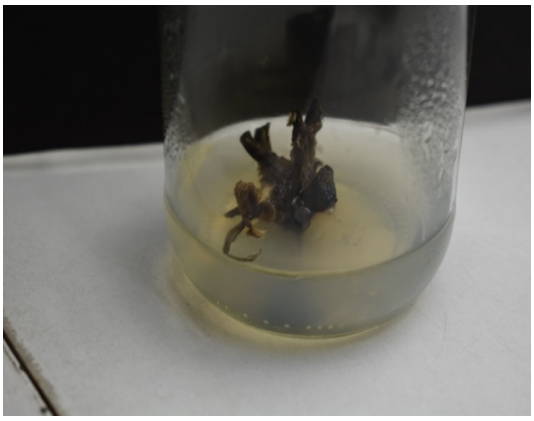
Initiation in T_{14} SPM Sor-2



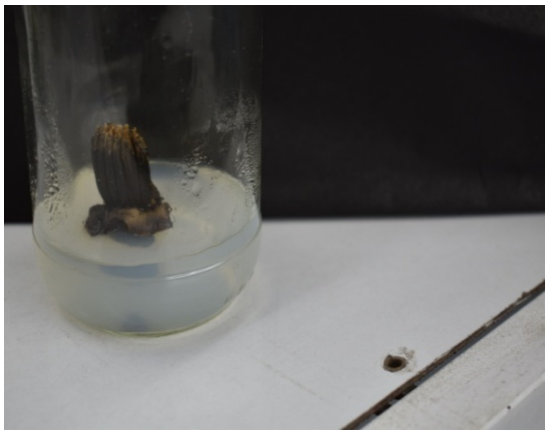
Initiation in T_{15} SPM Sor-3



Greening in T_{14} SPM Sor-2

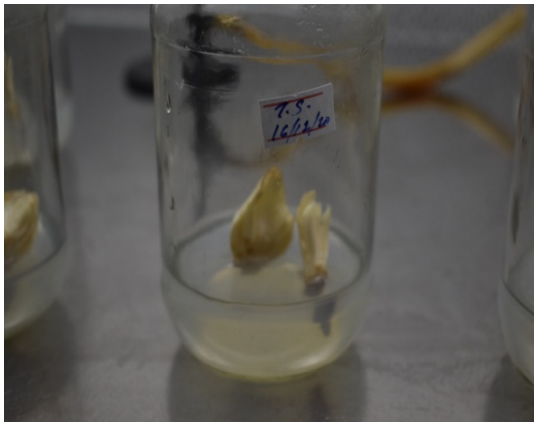


Blackening in T_{15} SPM Sor-3

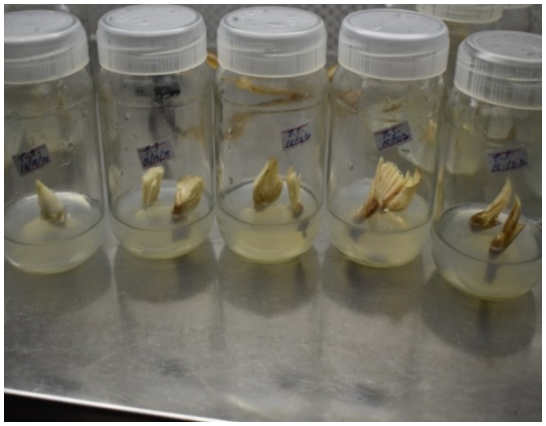


Blackening in T_{16} SPM Sor-4

Plate 16 Effect of Sorbitol in culture establishment of banana explants (MFBs) cv Chinichampa (AAB)

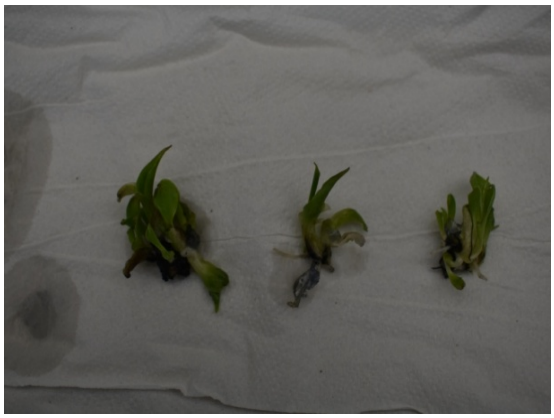


Initiation in T_{17} SPM TS-2



Initiation in T_{18} SPM TS-3

Subculturing in T_{17} SPM TS-2

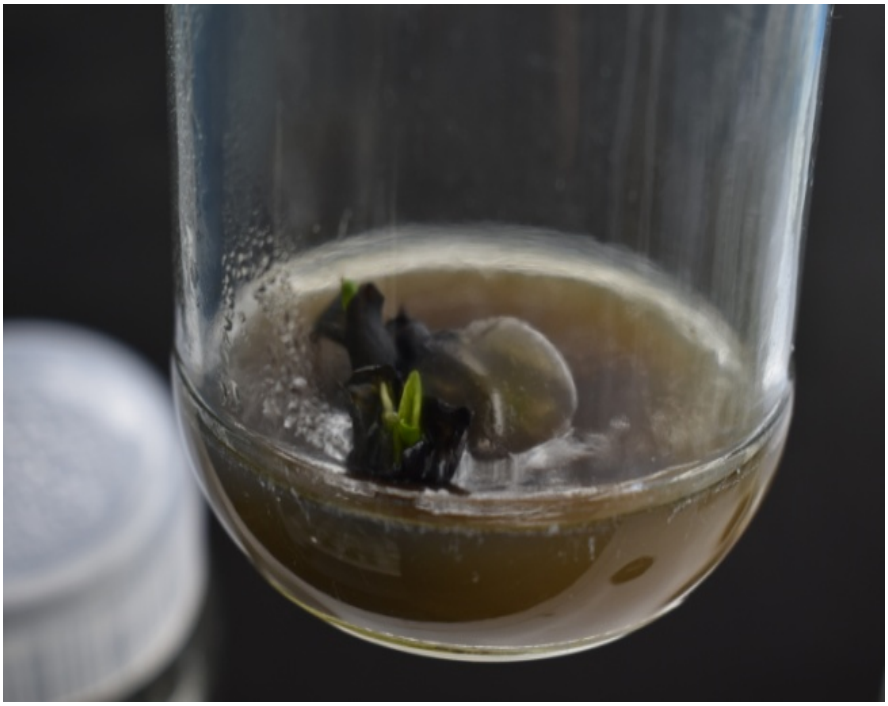


Subculturing in T_{18} SPM TS-3

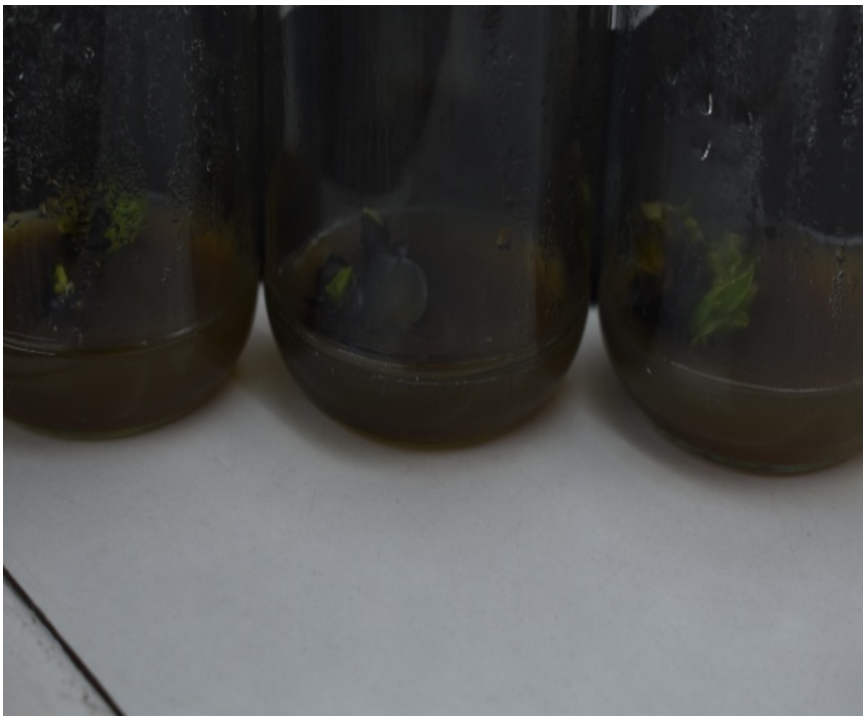
Elongation in T_{17} SPM TS-2

Elongation in T_{18} SPM TS-3

Plate 17 Effect of table sugar in culture establishment of banana explants (MFBs) cv Chinichampa (AAB)



Initiation in T_{20} SPM J-2



Initiation in T_{21} SPM J-3

Plate 18 Effect of Jaggery in culture establishment of banana explants (MFBs) cv Chinichampa (AAB)

4.3 Experiment – 3

In this experiment, the effect of various concentrations of carbon sources were tested for *in vitro* rooting of banana cv. Chinichampa (AAB) through shoot tips. The findings of the investigation has been properly documented and portrayed in table and graphically depicted through column chart.

4.3.1 Days taken for root initiation

The duration for root initiation was recorded in days when the micro shoots starts initiating roots after inoculation in rooting medium. The growth and development of *in vitro* culture is mostly affected by the concentrations of the carbon source rather than by the type of the carbon used (Mehta, 1980). Out of the various carbohydrates tested at different levels in the experiment, earliest rooting was found in T_3 (6.48 days) which were statistically at with T_2 (6.61 days), T_{18} (6.83 days), T_4 (7.00 days), T_{19} (7.00), T_{17} (7.13 days) and T_1 (7.14 days). While the maximum days recorded for root initiation was in T_{14} (14.00 days) and T_{15} (14.00 days). The root induction medium containing jaggery as carbon source became dry after 3 weeks of culture due to inhibitore present in the media.

Root initiation and growth are high energy demanding processes and can occur only at the expenses of available metabolic substrates (Hossain *et al*., 2009). Sucrose is broken down during autoclaving and converted to glucose and fructose by the action of invertase (Pierik, 1997). Glucose is then utilized first and followed by fructose. There are also reports whereby glucose has been found to be better source of carbon than sucrose (Hsaio and Korban, 1996; Sul and Korban, 1998; Cuenca and Vietiez, 2000). The failure of culture establishment in jaggery supplemented root induction medium may be due to adverse effect of jaggery on shoot and root growth as opined by Joshi *et al*. (2009) in micropropagation of *Wrightia tomentosa*. In a finding by Ahmed *et al*. (2014), it was observed that rooting in *Musa* cv. Grand Naine was

successfully initiated on media with 1 to 3% sucrose.

4.3.2 Rooting percentage (%)

The observation recorded for rooting percentage (%) has been presented below in table 4.3 and graphically depicted in figure 4.16. It was clearly evident from the table that 96% to 100% rooting was recorded in treatments T_{19} (100%), T_{18} (96.67%), T17 (96.67%) and T_1 (96.67%). Meanwhile treatments like T_{11} (6.67%), T_{10} (13.34%), T_{13} (10.00%), T_{15} (6.67%) and T_{16} (10%) exhibited low percentage of rooting. The least rooting percentage was found in T_{14} (3.34%). In the present experiment, no culture response was recorded in T_{20}, T_{21} and T_{22}.

This study also revealed that table sugar as carbon source at 20 g/l, 30 g/l and 40 g/l induces 96% to100% rooting. Similarly, sucrose at 30 g/l induced 96.67% rooting. This might be attributed to the presence of 96-97% sucrose in table sugar (Tyagi *et al*., 2007). Similar finding has been reported by Ahmed *et al*. (2014) in Grand Naine banana where 3% sucrose induced 100% rooting. In *Withania somnifera,* 100% root induction was achieved by concentration of 3% sucrose (Sivanesan and Murugesan, 2008). In micropropagation of common Ninebark, Ilczuk *et al*. (2013) revealed that fructose stimulated higher level of rooting and rooting degree. The carbohydrate requirements in *in vitro* propagation not only depend upon the stages of culture but may also vary from species to species (Thompson and Thorpe, 1987).

4.3.3 Number of roots per shoot

As evident from the table 4.3 there was significant difference in number of roots per shoot as influence by different carbon sources. It was recorded that treatment T_{18} (4.59) exhibit maximum number of roots per shoot which was statistically at par with T_1 (4.58); followed by T_{12} (4.42) which was again at par with T_{17} (4.38). The minimum number of roots per shoot was recorded in treatment T_{16} (2.83). Similar finding has been reported by Prabhuling and

Sathyanarayan (2017) in Grand Naine banana cultivar where 30 g/l common grade sugar was found superior in *in vitro* rooting; it may be because of their efficient translocation and assimilation by the explants resulting in enhanced cell division and eventual growth. More findings were also reported in banana (Kodym and Zapata-Arias, 2001; Saeed, 2006 and Das and Gupta, 2009), ginger (Sharma and Singh, 1995), anthurium (Prabhakara, 1999), strawberry (Kaur *et al*., 2005) and *Centella asiatica* (Raghu *et al*., 2007). While Romano *et al*. (1995) has found that sorbitol failed to simulate shoot proliferation and rooting in cork oak. Similarly results were found in the present investigation in the response of plantlets cultured in sorbitol media. As a result it can be deduced that sorbitol is less effective in *Musa* sp. as it is not efficiently metabolized; but help regulates osmotic potential only.

4.3.4 Length of longest roots (cm) after 30 days

In the experiment, the length of the longest root in each treatment was measured with the help of graduated scale from the base of the shoot to the tip of the roots and average was expressed in centimeters. The data obtained with regards to length of longest roots (cm) after 30 days has been presented below in table 4.3 and figure 4.18. The longest root was observed in T_{17} (7.74 cm); it was followed by T_{18} (7.51 cm) which was statistically at par with and T_1 (7.44 cm) and T_{19} (7.15 cm). The shortest roots length was recorded in T_{14} (4.10 cm).

Table sugar contains 96-97% sucrose as compare to 99.98% in laboratory grade sucrose (Tyagi *et al*., 2007). The present findings revealed insignificance difference on length of roots as affected by sucrose and table sugar as carbon source. Similar results were obtained in Basrai banana (Memon *et al*., 2019), Sabri banana (Hossain *et al*., 2009), Grand Naine (Ahmed *et al*., 2014b) and Japanese Pear (Kadota and Niimi, 2004).

4.3.5 Average length of shoot (cm) after 30 days

The observation taken for average length of shoot (cm) after 30 days has

been given in table 4.3 and figure 4.19. The length of all the rooted shoots were measured from the base of the collar region to tip of the longest leaves and expressed in centimeters. The maximum length of shoot recorded was in T_4 (6.93 cm) followed by T_{18} (6.71 cm) which was statistically at par with T_{19} (6.64 cm) and T_{17} (6.57 cm). Treatment T_8 (3.98 cm) recorded the minimum average length of shoot.

Shoot regeneration and elongation in *in vitro* culture depends considerably with the nutrients constituents of the culture media (Chen *et al*., 2003). In general, sucrose is commonly used in micropropagation to induce adventitious shoots and buds. According to some studies glucose has been found to be better source of carbon than sucrose (Hsaio and Korban, 1996; Sul and Korban, 1998; Cuenca and Vietiez, 2000). In the present investigation, it was revealed that glucose at 40 g/l and table sugar at 20, 30, and 40 g/l induced the maximum shoot length. Sudipta *et al*. (2003) found that *Leptadenia reticulate* shoots induced on MS medium supplemented with 2% table sugar results in maximum shoot length. Similarly, Prabhuling and Sathyanarayana (2017) found commercial sugar at 30 g/l superior for shoot multiplication in Grand Naine. However, Memon *et al*. (2019) obtained maximum shoot length in Basrai banana with 30 g/l sucrose. Similar finding were recorded by Swetha and Srinivas in *Oxalis corniculata* (L.). But in case of *in vitro* regeneration in *Solanum viarum,* fructose at 4% induced maximum mean shoot length.

4.3.6 Number of leaves per plantlets

In the present experiment the total number of leaves formed per rooted shoots was recorded and expressed in average (Table 4.3 and figure 4.20). The highest number of leaves per plantlets was observed in T_1 (4.56) followed by T_{18} (4.51) which was statistically at par with T_{17} (4.41) and T_{19} (4.40). The lowest number of leaves was observed in T_{14} (3.00). Similar findings were reported by Ahmed *et al*. (2014b) in Grand Naine and Agrawal *et al*. (2010) in

Karpura Chakkarakeli banana. However in strawberry, the highest number of leaves was recorded in media supplemented with glucose (Abdullah *et al*., 2013).

Table 4.3 Effect of modified MS medium supplemented with various concentrations of carbon sources on *in vitro* rooting of banana cv. Chinichampa (AAB) through shoot tips

Treatments	Days taken for root initiation	Rooting percentage (%)	Number of roots per shoot	Length of longest roots (cm) after 30 days	Average length of shoot (cm) after 30 days	Number of leaves per plantlets
T_1: RIM S	7.14^{e}	96.67	4.58^{a}	7.44^{ab}	6.34^{ab}	4.56^{a}
T_2: RIM G2	6.61^{e}	46.67	3.48^{cd}	6.77^{bc}	6.00^{bc}	3.89^{c}
T_3: RIM G3	6.48^{e}	50.00	3.71^{cd}	6.79^{bc}	6.43^{ab}	4.14^{ab}
T_4: RIM G4	7.00^{e}	40.00	3.57^{cd}	6.83^{bc}	6.93^{a}	4.00^{bc}
T_5: RIM F2	7.92^{d}	26.67	3.25^{de}	5.26^{ef}	4.87^{d}	3.50^{cd}
T_6: RIM F3	8.23^{de}	40.00	3.23^{de}	6.13^{cd}	5.44^{cd}	4.25^{ab}
T_7: RIM F4	8.80^{d}	30.00	3.13^{e}	5.84^{de}	6.07^{bc}	4.00^{bc}
T_8: RIM Man-2	8.45^{de}	40.00	4.14^{ab}	6.60^{c}	3.98^{f}	3.53^{cd}
T_9: RIM Man-3	7.25^{e}	33.34	4.08^{ab}	6.49^{cd}	4.06^{f}	3.83^{c}
T_{10}: RIM Man-4	7.83^{de}	13.34	4.00^{bc}	5.68^{ef}	5.00^{de}	4.00^{bc}
T_{11}: RIM Mal-2	11.67^{b}	6.67	3.17^{e}	5.22^{ef}	4.50^{ef}	3.17^{de}
T_{12}: RIM Mal-3	8.58^{d}	26.67	4.42^{ab}	6.34^{cd}	5.12^{de}	4.17^{abc}
T_{13}: RIM Mal-4	10.00^{c}	10.00	3.33^{de}	7.02^{ab}	5.22^{de}	4.33^{ab}
T_{14}: RIM Sor-2	14.33^{a}	3.34	3.00^{e}	4.10^{g}	4.75^{e}	3.00^{d}
T_{15}: RIM Sor-3	12.83^{b}	6.67	3.33^{de}	5.00^{f}	4.95^{de}	3.50^{cd}
T_{16}: RIM Sor-4	14.33^{a}	10.00	2.83^{e}	5.60^{e}	5.53^{cd}	3.50^{cd}
T_{17}: RIM TS-2	7.13^{e}	96.67	4.38^{ab}	7.74^{a}	6.57^{ab}	4.41^{ab}
T_{18}: RIM TS-3	6.83^{e}	96.67	4.59^{a}	7.51^{ab}	6.71^{ab}	4.51^{ab}
T_{19}: RIM TS-4	7.00^{e}	100.00	4.03^{bc}	7.15^{ab}	6.64^{ab}	4.40^{ab}
T_{20}: RIM J-2	0.00^{f}	0.00	0.00^{f}	0.00^{h}	0.00^{g}	0.00^{e}
T_{21}: RIM J-3	0.00^{f}	0.00	0.00^{f}	0.00^{h}	0.00^{g}	0.00^{e}
T_{22}: RIM J-4	0.00^{f}	0.00	0.00^{f}	0.00^{h}	0.00^{g}	0.00^{e}
SEm±	0.41	-	0.16	0.24	0.22	0.16
CD (P=0.05)	1.18	-	0.45	0.68	0.63	0.45

*Means with different superscript letters within column are significantly different from each other by DMRT (*p=0.05*)

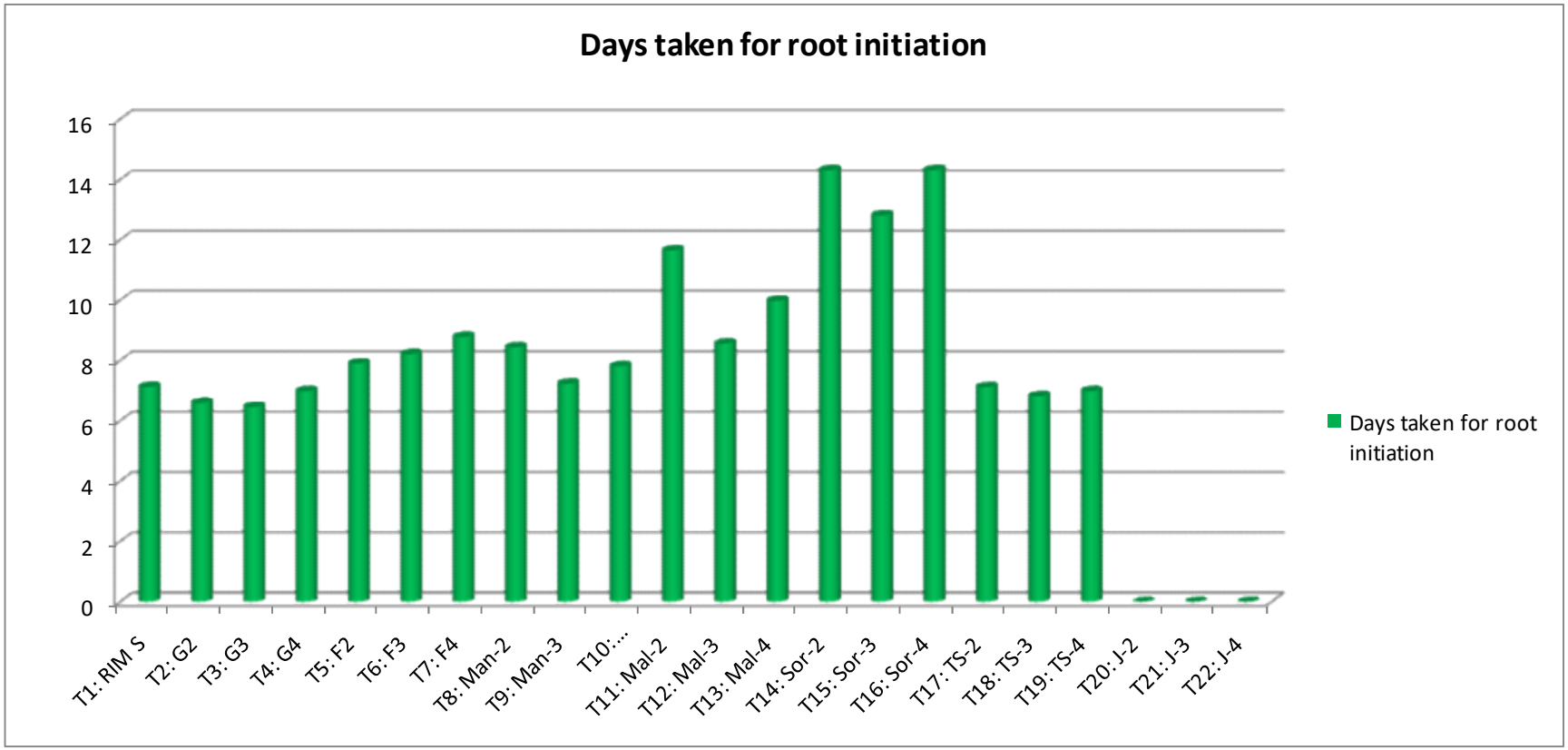


Figure 4.15 Effect of modified MS medium supplemented with various concentrations of carbon sources on days taken for root initiation on *in vitro* rooting of banana cv. Chinichampa (AAB) through shoot tips

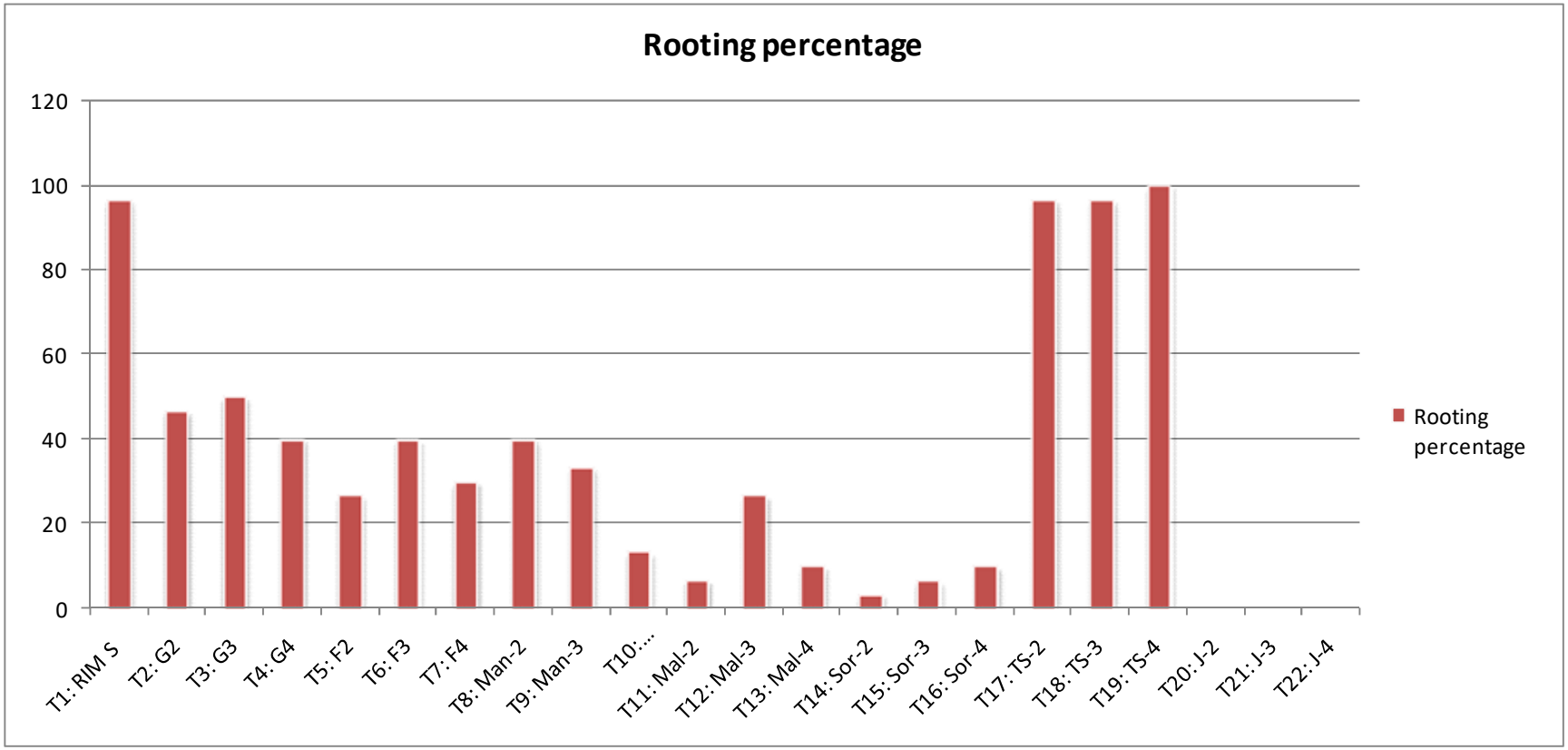


Figure 4.16 Effect of modified MS medium supplemented with various concentrations of carbon sources on rooting percentage of *in vitro* rooting of banana cv. Chinichampa (AAB) through shoot tips

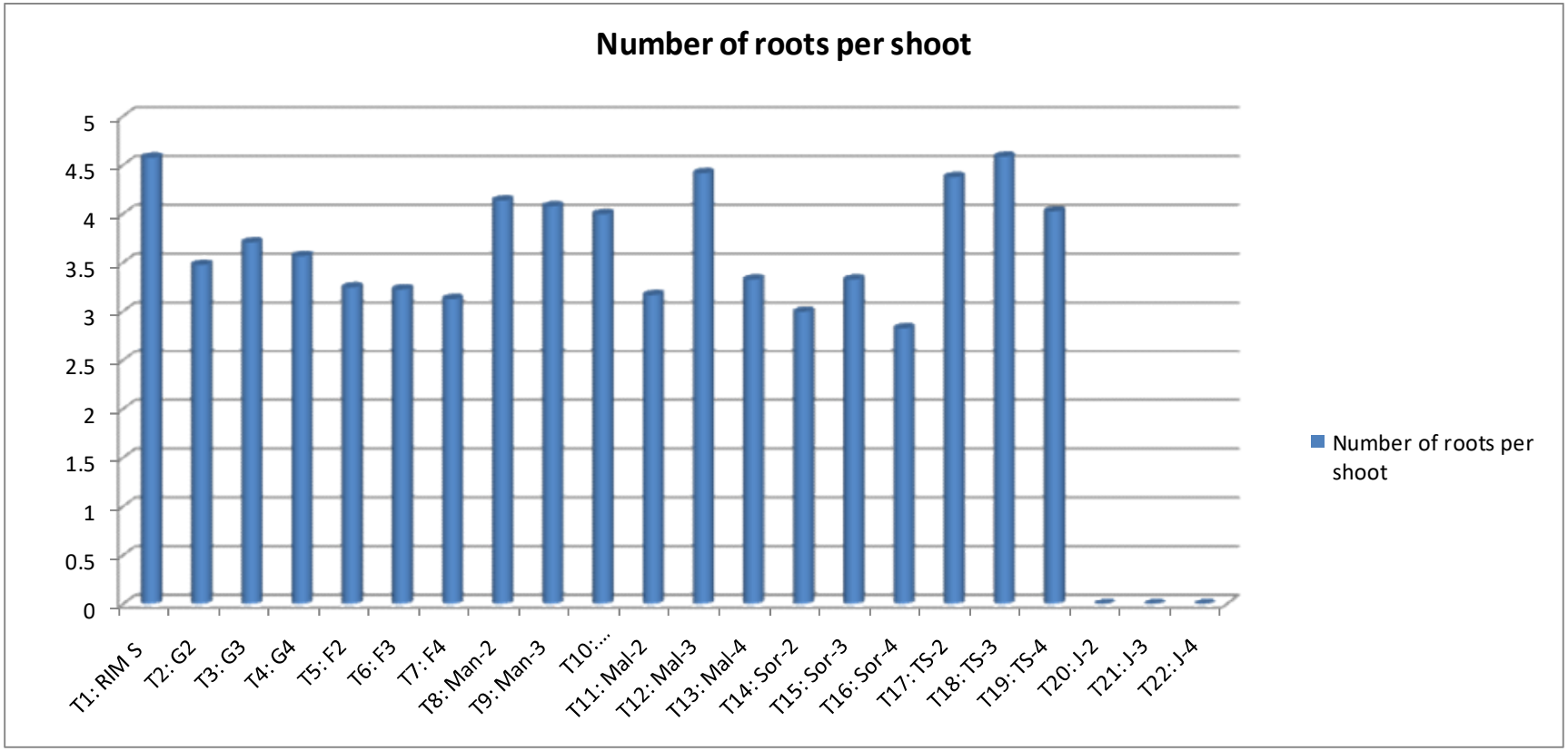


Figure 4.17 Effect of modified MS medium supplemented with various concentrations of carbon sources on rooting percentage of *in vitro* rooting of banana cv. Chinichampa (AAB) through shoot tips

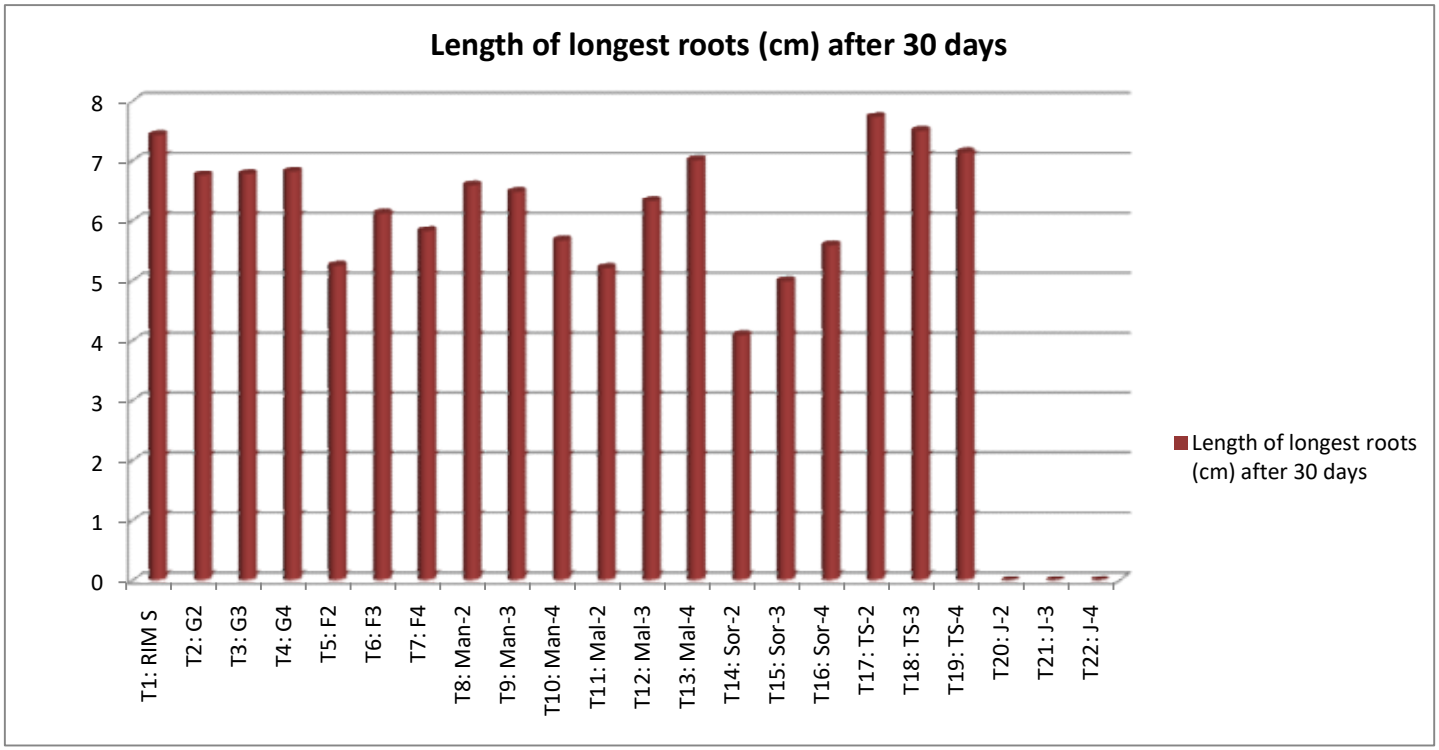


Figure 4.18 Effect of modified MS medium supplemented with various concentrations of carbon sources on length of longest roots (cm) after 30 days in *in vitro* rooting of banana cv. Chinichampa (AAB) through shoot tips

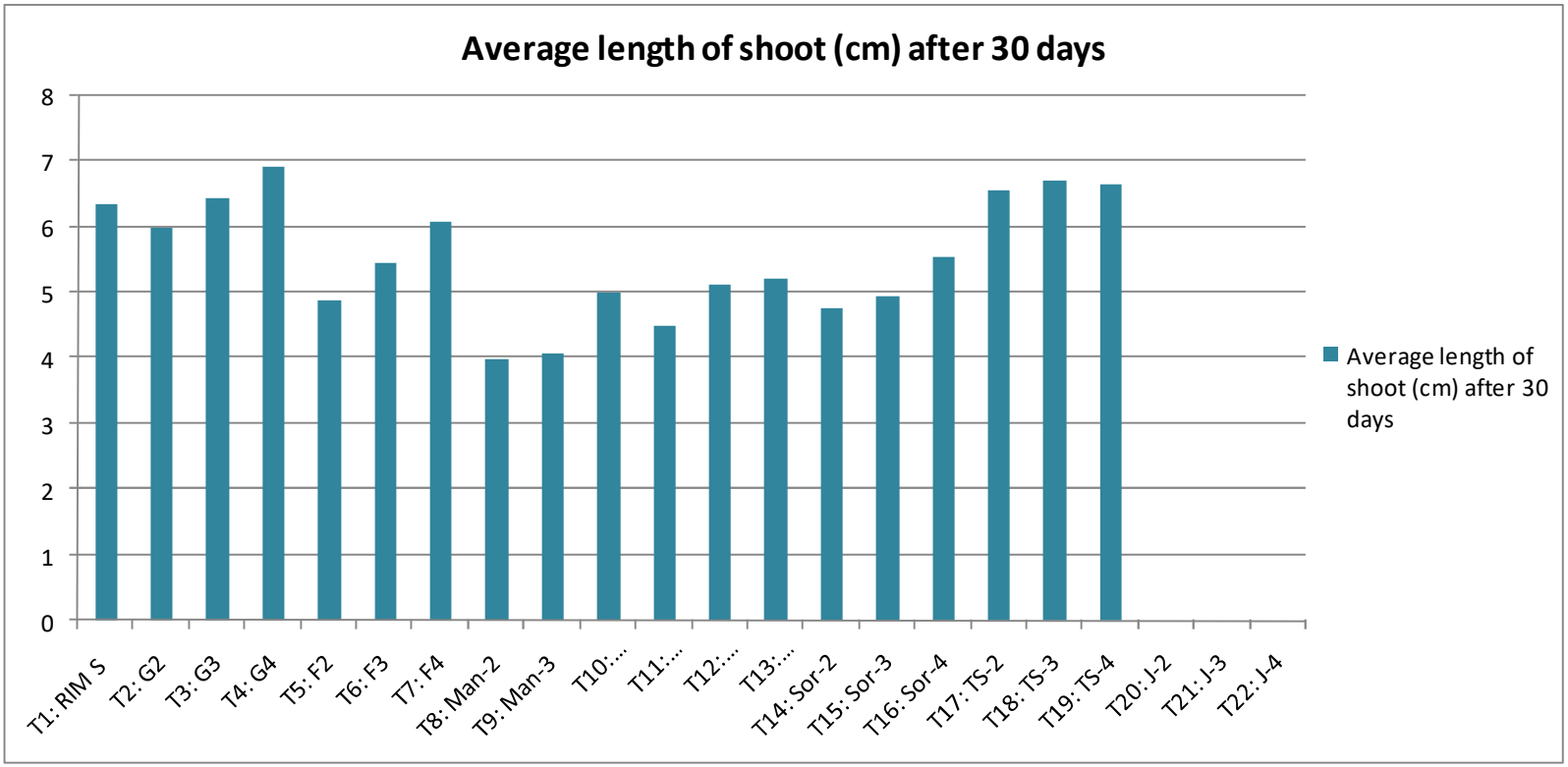


Figure 4.19 Effect of modified MS medium supplemented with various concentrations of carbon sources on average length of shoot (cm) after 30 days in *in vitro* rooting of banana cv. Chinichampa (AAB) through shoot tips

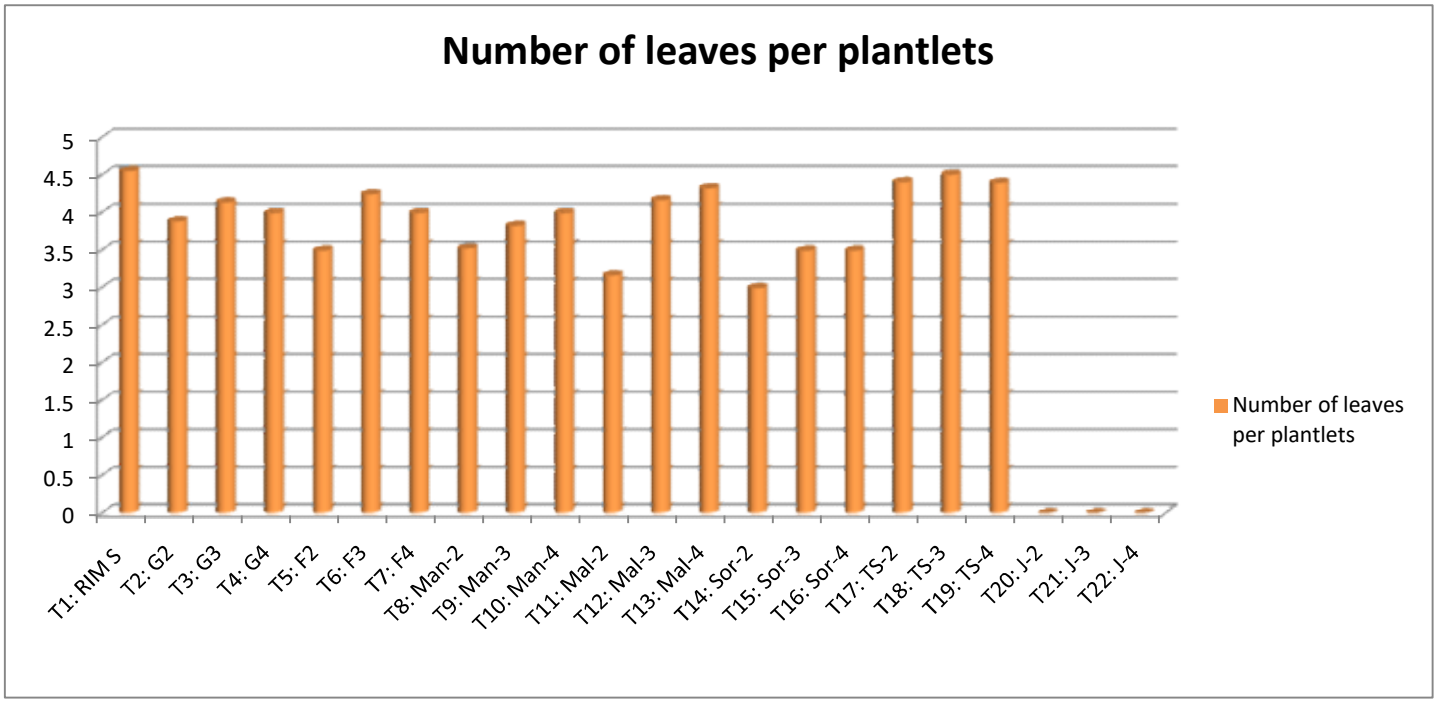
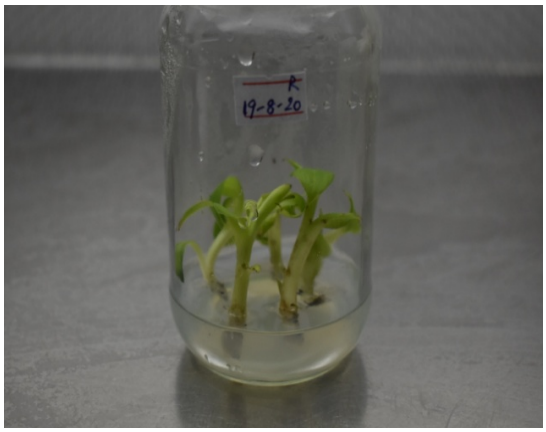
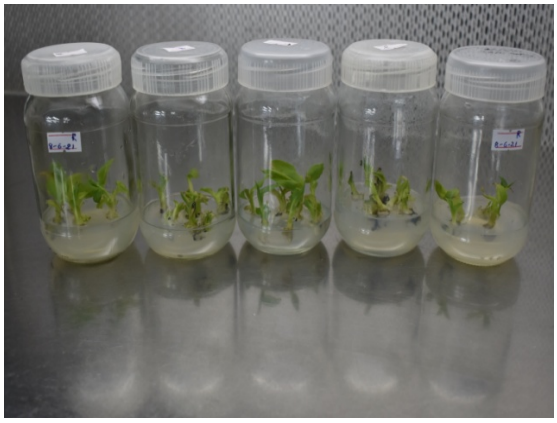


Figure 4.20 Effect of modified MS medium supplemented with various concentrations of carbon sources on number of leaves per plantlets in *in vitro* rooting of banana cv. Chinichampa (AAB) through shoot tips

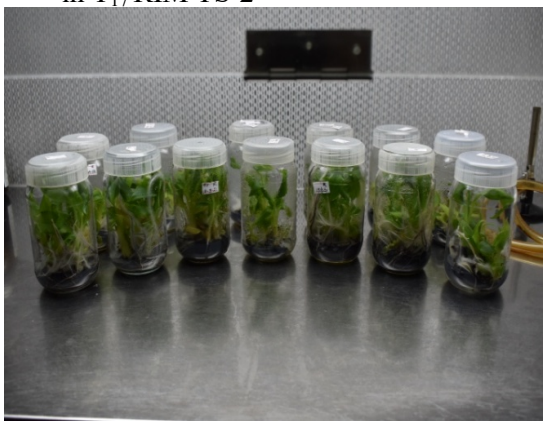
Isolation of elongated shoots



Initiation of shoots for rooting in T_{17} RIM TS-2

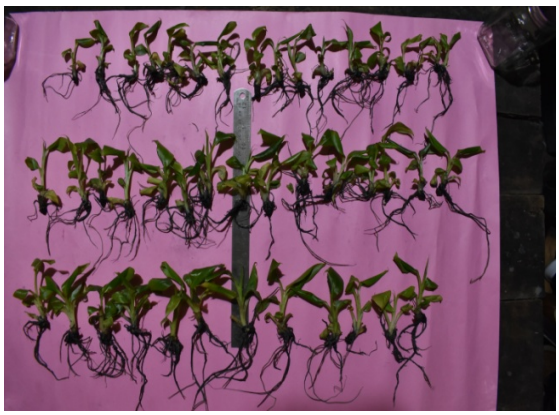


Initiation of shoots for rooting in T_{18} RIM TS-3



Rooting in T_{18} RIM TS-3

Estimatation of shoots length in T_{18} RIM TS-3



Measurement of root length in in T_{18} RIM TS-3

Plate 19 Effect of Table sugar 30 g/l in rooting of banana explants (shoot tip) cv Chinichampa (AAB)

4.4 Experiment – 4

In this experiment, the effects of various concentrations of eight different carbon sources were tested for *in vitro* rooting of banana cv. Chinichampa (AAB) through immature male floral buds (MFBs). The findings of the investigation has been properly documented and portrayed in table and graphically depicted through column chart.

4.4.1 Days taken for root initiation

The data relevant to days taken for root initiation has been presented in table 4.4 and figure 4.21. From the data recorded it was evident that the minimum days required for root initiation was recorded in T_{18} (7.60) which was statistically at par with T_1 (7.73) followed by T_3 (7.88) which was again at par with T_{19} (7.97). Maximum duration for root initiation was observed in T_{11} (13.33) while T_{14}, T_{15}, T_{20}, T_{21} and T_{22} failed to produce any roots. The study revealed the effectiveness of table sugar and sucrose in root initiation and subsequent growth and development of the explants. Root initiation and growth are high energy demanding processes and can occur only at the expenses of available metabolic substrates (Hossain *et al*., 2009). It is also a known fact that table sugar contains 96-97% sucrose (Tyagi *et al*., 2007). The failure of culture establishment in jaggery supplemented root induction medium may be due to adverse effect of jaggery on shoot and root growth as opined by Joshi *et al*. (2009) in micropropagation of *Wrightia tomentosa*.

4.4.2 Rooting percentage (%)

As evident from table 4.4 and figure 4.22, 100% rooting was recorded in T_1 and T_{18}. High percentage of rooting was also observed in T_{19} (96.67%) and T_{17} (90.00%). Negligible percentage of rooting was observed in T_{16} (6.67%). The study revealed that table sugar as carbon source at 20 g/l, 30 g/l and 40 g/l induces 90.00% to100% rooting. Similarly, sucrose at 30 g/l induced 100.00% rooting. This might be attributed to the presence of 96-97% sucrose in table

sugar (Tyagi *et al*., 2007). For most plant tissue culture protocols, sucrose is the preferred carbon source as it is an easily assimilated macronutrient that rapidly provides energy (Saraswathi *et al*., 2016).

4.4.3 Number of roots per shoot

The data pertaining to number of roots per shoot has been presented in table 4.4 and graphically in figure 4.23. The maximum number of roots per shoot was observed in T_1 (4.87) which were at par with T_{18} (4.77) followed by T_{19} (4.55) and T_{17} (4.47) which were both statistically at par. While the lowest number of roots per shoot was recorded in T_{13} (3.33). Most of the treatments in the experiment showed almost similar number of roots per shoot. Prabhuling and Sathyanarayan (2017) observed that common grade sugar was found superior in *in vitro* rooting because of its efficient translocation and assimilation by the explants resulting in enhanced cell division and eventual growth. Nair *et al*. (2018) obtained a mean average of 6.25 roots per shoot in Poovan banana from direct shoot regeneration of immature male flower buds in a medium containing 1 mg/l BAP. Similar result has been replicated by Kavitha *et al*. (2020) in Rasthali banana in a medium containing 1 mg/l IBA and NAA.

4.4.4 Length of longest roots (cm) after 30 days

For estimating the length of the longest roots a graduated scale was used and the longest visible root was measured from base of the shoot to the tip of the root and average was expressed in centimeters. The data obtained with regards to length of longest roots (cm) after 30 days has been shown below in table 4.4 and figure 4.24. The longest root was observed in T_{18} (6.68 cm) which was statistically at par with T_{19} (6.55 cm), T_1 (6.54 cm) and T_{17} (6.25 cm). Meanwhile, shortest length of root was observed in T_{16} (4.10 cm). The present findings revealed insignificance difference on length of roots as affected by sucrose and table sugar as carbon source. Table sugar contains 96-97% sucrose as compare to 99.98% in laboratory grade sucrose (Tyagi *et al*., 2007). Kavitha *et al*. (2020) and Nair *et al*. (2018) obtained 12.10 cm and 5.87 cm root length

from *in vitro* propagation of immature male buds in a medium containing 1 mg/l NAA and IBA respectively.

4.4.5 Average length of shoot (cm) after 30 days

The observation taken for average length of shoot (cm) after 30 days has been shown in table 4.4 and figure 4.25. It is evident from the data that the highest average length of shoot (cm) was observed in T_{18} (6.34 cm) which was statistically at par with T_1 (6.31 cm), T_{17} (6.15 cm) and T_{19} (6.01 cm). The treatment T_4 recorded an average length of 5.27 cm. Meanwhile shortest average length of shoot was recorded in T_{16} at 3.31 cm. Resmi and Nair (2007) obtained a shoot length of 4.91 cm and 6.50 cm after 60 days from micropropagation of male flower buds of Sannachenkadali and Red Banana banana respectively in a medium containing 8.9 µM BA.

4.4.6 Number of leaves per plantlets

The data relevant to number of leaves per plantlets has been presented in table 4.4 and figure 4.26. The highest number of leaves per plantlets was observed in T_1 (5.08) which were statistically at par with T_{18} (4.89) followed by T_{19} (4.79), T_{17} ((4.66) which were both at par and T_4 (4.36). While the least number of leaves per plantlets was observed in T_{11} (3.00). Similar finding was reported by Kavitha *et al.* (2020) in mass multiplication Rasthali banana through male floral buds in a medium containing 1 mg/l NAA. Higher number of leaves ultimately results in better survival rate and initial establishment in the nursery as reported earlier by Mahadev *et al.* (2011).

Table 4.4 Effect of modified MS medium supplemented with various concentrations of carbon sources on *in vitro* rooting of banana cv. Chinichampa (AAB) through male floral buds

Treatments	Days taken for root initiation	Rooting percentage (%)	Number of roots per shoot	Length of longest roots (cm) after 30 days	Average length of shoot (cm) after 30 days	Number of leaves per plantlets
T_1: RIM S	7.73^{e}	100.00	4.87^{a}	6.45^{a}	6.31^{a}	5.08^{a}
T_2: RIM G2	8.36^{de}	53.34	3.61^{c}	4.77^{bc}	3.93^{e}	3.62^{def}
T_3: RIM G3	7.88^{d}	56.67	3.92^{cd}	4.89^{bc}	4.89^{bc}	4.01^{cd}
T_4: RIM G4	8.58^{de}	36.67	3.61^{cd}	4.28^{c}	5.27^{b}	4.36^{bc}
T_5: RIM F2	11.67^{bc}	40.00	3.67^{cd}	4.19^{c}	4.45^{cd}	3.36^{efg}
T_6: RIM F3	10.50^{c}	46.67	3.52^{cd}	4.53^{bc}	4.66^{cd}	3.83^{de}
T_7: RIM F4	11.39^{bc}	30.00	3.58^{cd}	4.56^{bc}	4.86^{bc}	3.81^{de}
T_8: RIM Man-2	9.17^{d}	33.34	3.75^{cd}	4.89^{bc}	3.95^{e}	3.20^{fg}
T_9: RIM Man-3	8.39^{de}	46.67	3.72^{cd}	5.32^{bc}	4.36^{cd}	3.95^{cd}
T_{10}: RIM Man-4	10.61^{bc}	23.34	4.00^{bc}	5.03^{b}	4.45^{cd}	3.83^{de}
T_{11}: RIM Mal-2	13.33^{a}	13.34	3.67^{cd}	4.77^{bc}	4.20^{de}	3.00^{g}
T_{12}: RIM Mal-3	11.17^{b}	26.67	3.78^{cd}	5.20^{b}	4.03^{de}	3.58^{def}
T_{13}: RIM Mal-4	11.83^{b}	23.34	3.33^{d}	4.73^{bc}	4.22^{de}	3.67^{def}
T_{14}: RIM Sor-2	0.00^{f}	0.00	0.00^{e}	0.00^{d}	0.00^{g}	0.00^{h}
T_{15}: RIM Sor-3	0.00^{f}	0.00	0.00^{e}	0.00^{d}	0.00^{g}	0.00^{h}
T_{16}: RIM Sor-4	14.33^{a}	6.67	3.50^{cd}	4.10^{c}	3.31^{f}	3.17^{fg}
T_{17}: RIM TS-2	8.27^{de}	90.00	4.47^{ab}	6.25^{a}	6.15^{a}	4.66^{ab}
T_{18}: RIM TS-3	7.60^{e}	100.00	4.77^{a}	6.68^{a}	6.34^{a}	4.89^{a}
T_{19}: RIM TS-4	7.97^{d}	96.67	4.55^{ab}	6.55^{a}	6.01^{a}	4.79^{ab}
T_{20}: RIM J-2	0.00^{f}	0.00	0.00^{e}	0.00^{d}	0.00^{g}	0.00^{h}
T_{21}: RIM J-3	0.00^{f}	0.00	0.00^{e}	0.00^{d}	0.00^{g}	0.00^{h}
T_{22}: RIM J-4	0.00^{f}	0.00	0.00^{e}	0.00^{d}	0.00^{g}	0.00^{g}
SEm±	*0.41*	-	*0.19*	*0.27*	*0.19*	*0.16*
CD (P=0.05)	*1.17*	-	*0.54*	*0.76*	*0.54*	*0.45*

*Means with different superscript letters within column are significantly different from each other by DMRT (*p=0.05*)

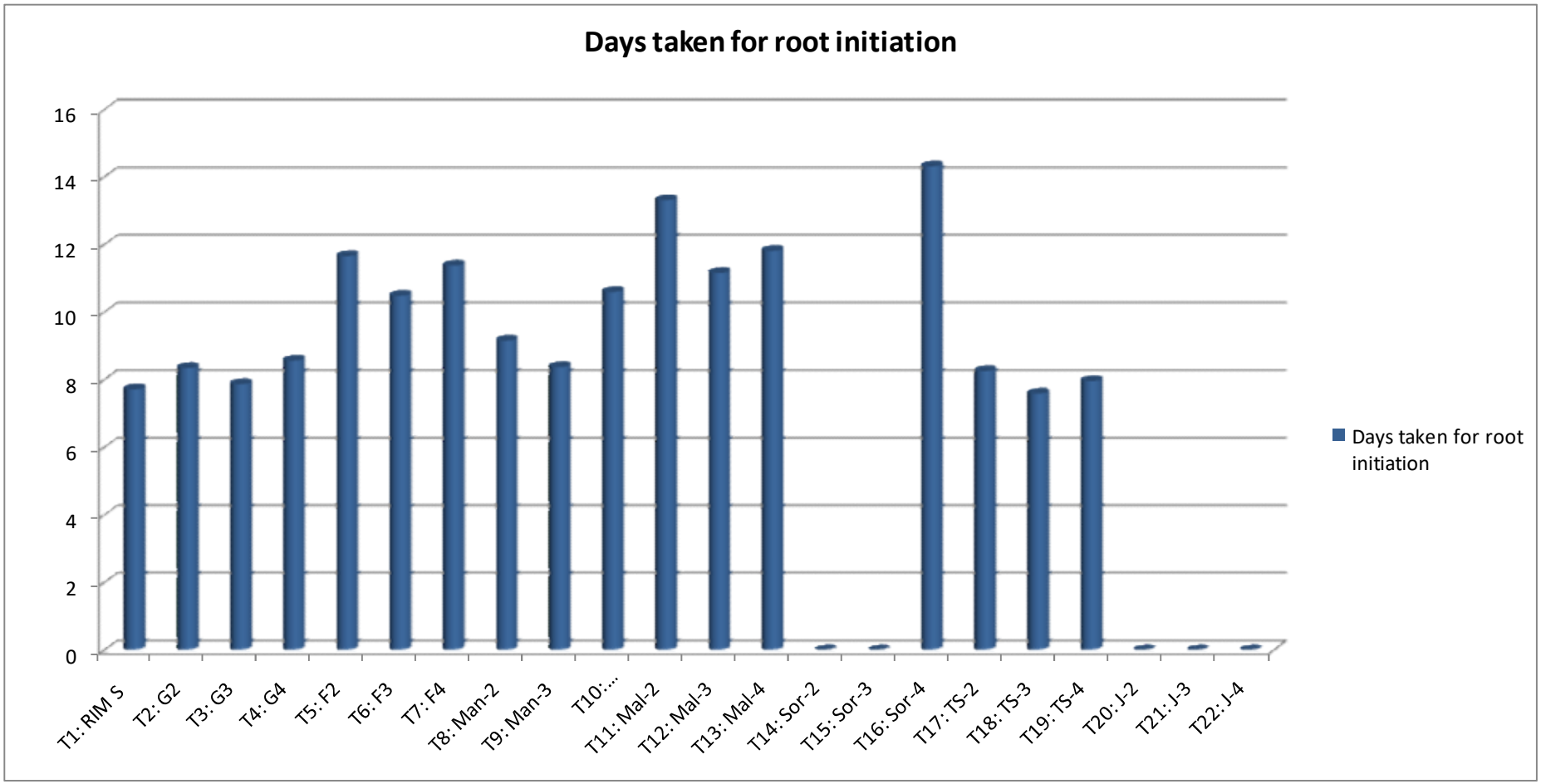


Figure 4.21 Effect of modified MS medium supplemented with various concentrations of carbon sources on days taken for root initiation in *in vitro* rooting of banana cv. Chinichampa (AAB) through male floral buds

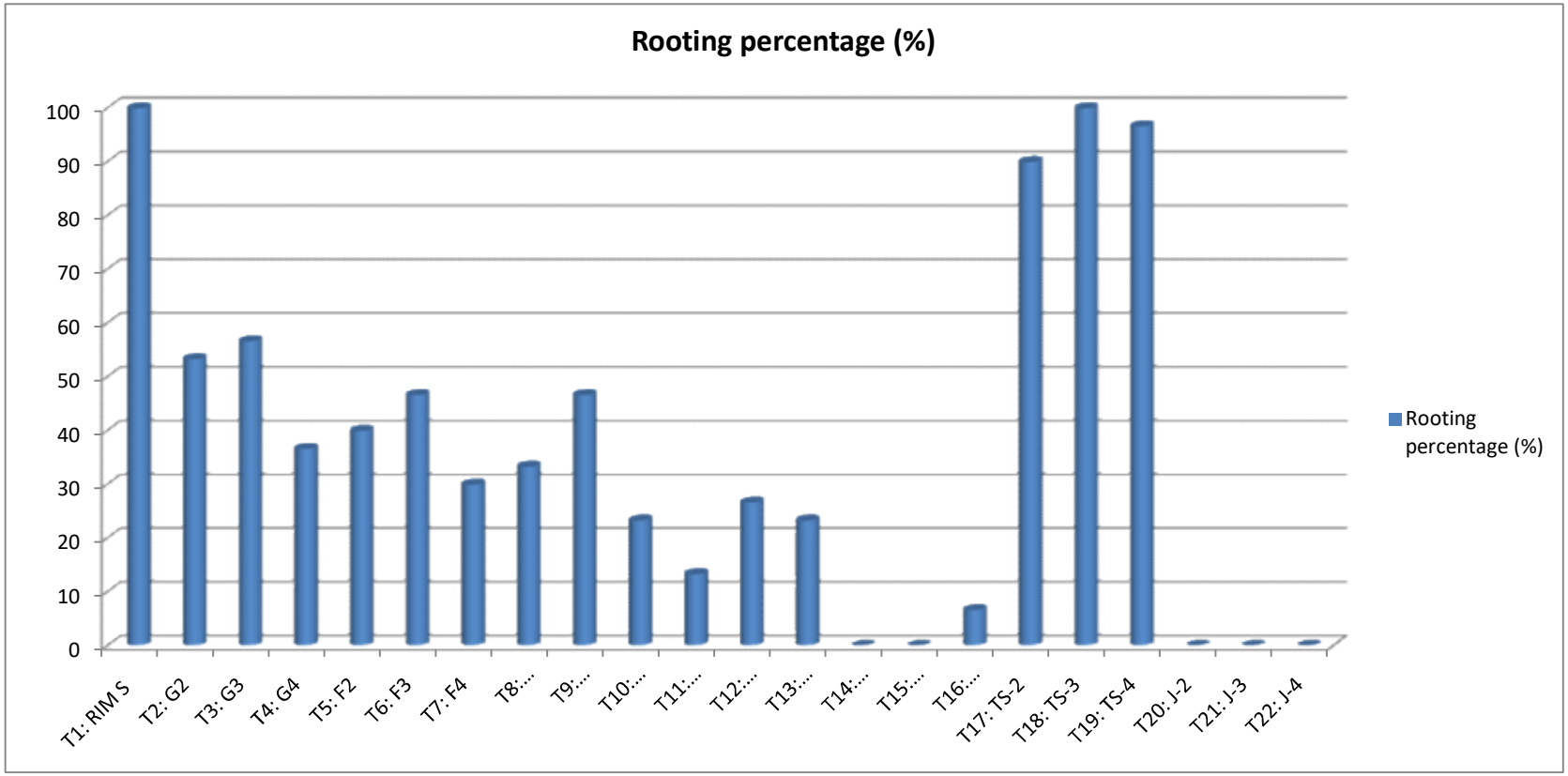


Figure 4.22 Effect of modified MS medium supplemented with various concentrations of carbon sources on rooting percentage (%) in *in vitro* rooting of banana cv. Chinichampa (AAB) through male floral buds

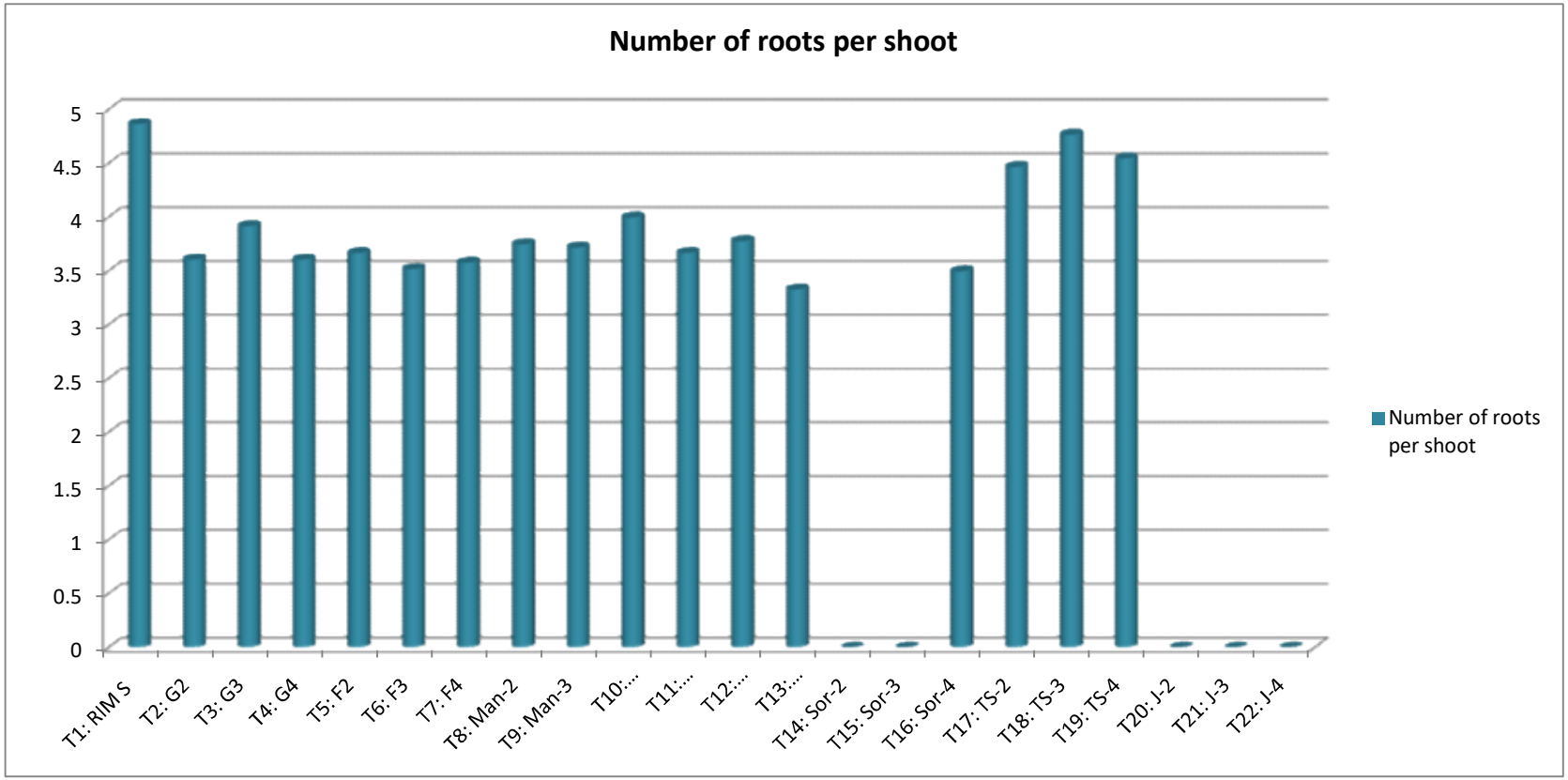


Figure 4.23 Effect of modified MS medium supplemented with various concentrations of carbon sources on number of roots per shoot in *in vitro* rooting of banana cv. Chinichampa (AAB) through male floral buds

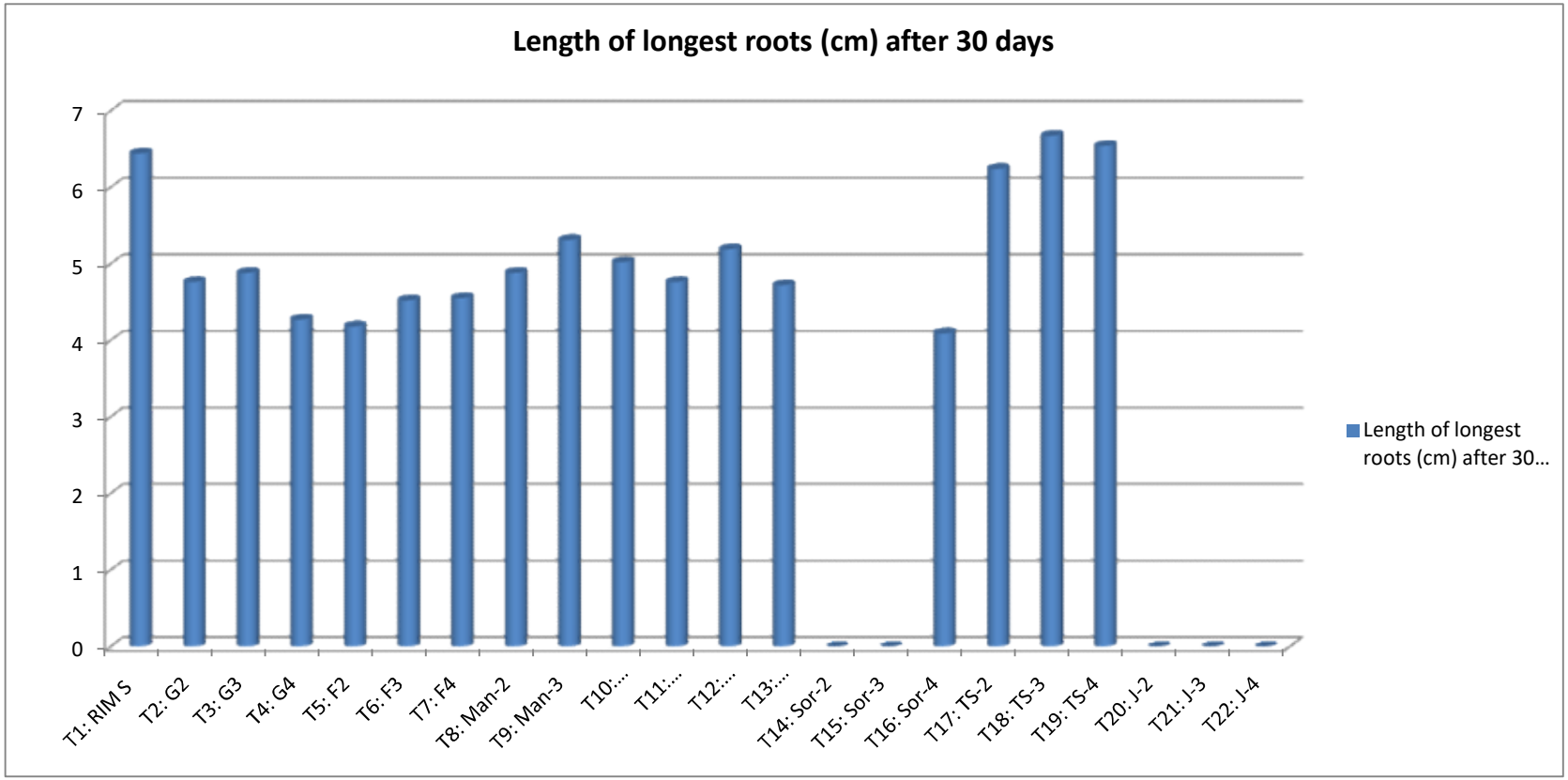


Figure 4.24 Effect of modified MS medium supplemented with various concentrations of carbon sources on length of longest roots after 30 days in *in vitro* rooting of banana cv. Chinichampa (AAB) through male floral buds

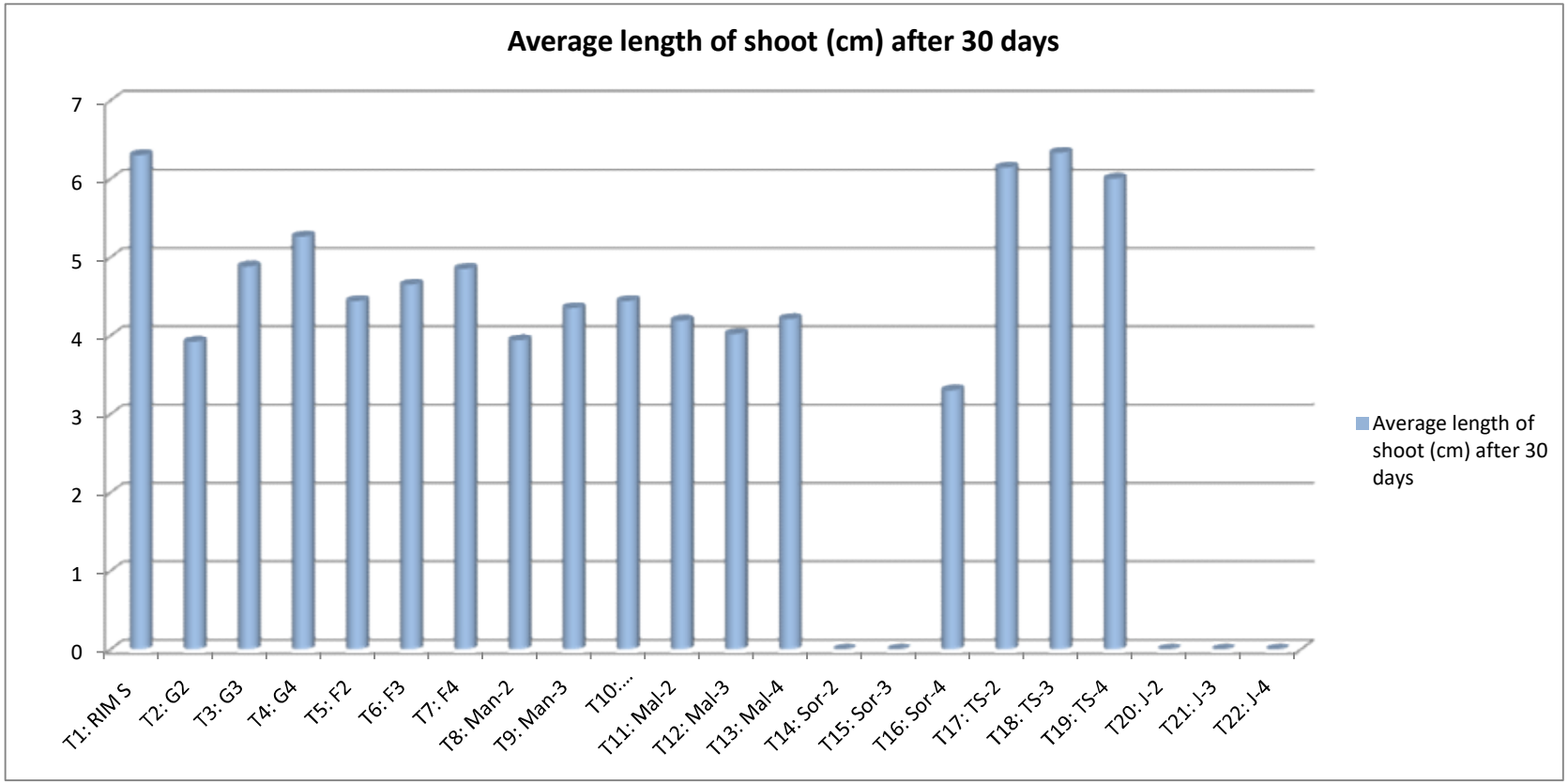


Figure 4.25 Effect of modified MS medium supplemented with various concentrations of carbon sources on average length of shoot after 30 days in *in vitro* rooting of banana cv. Chinichampa (AAB) through male floral buds

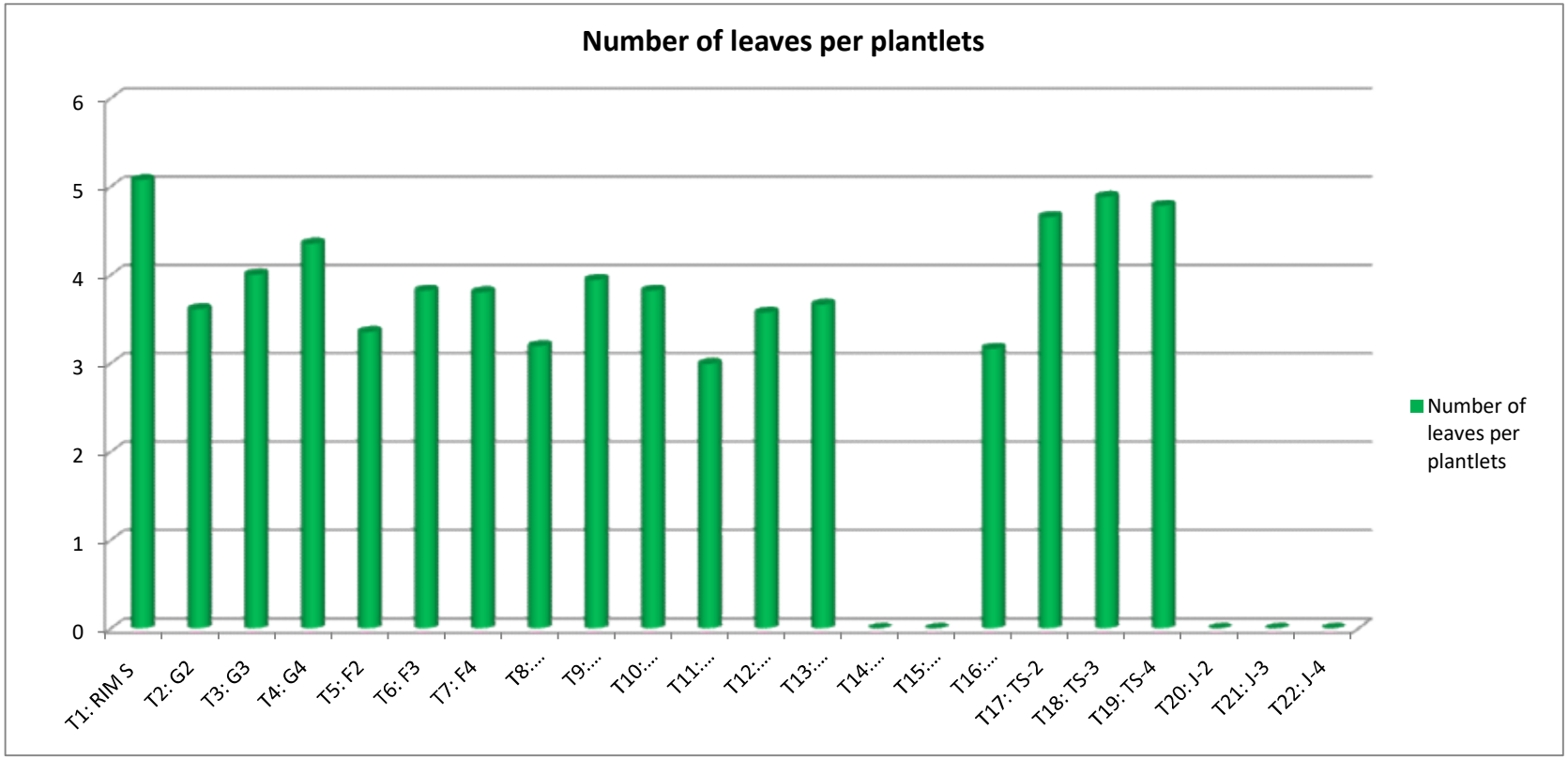
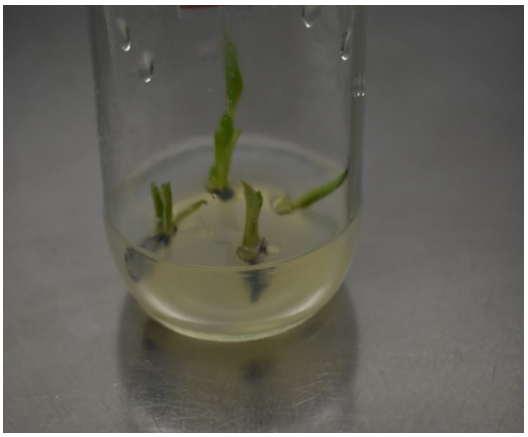
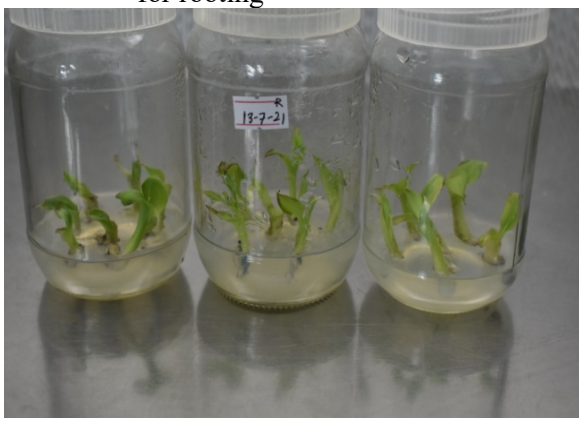


Figure 4.26 Effect of modified MS medium supplemented with various concentrations of carbon sources on number of leaves per plantlets in *in vitro* rooting of banana cv. Chinichampa (AAB) through male floral buds

Isolation of elongated shoots for rooting

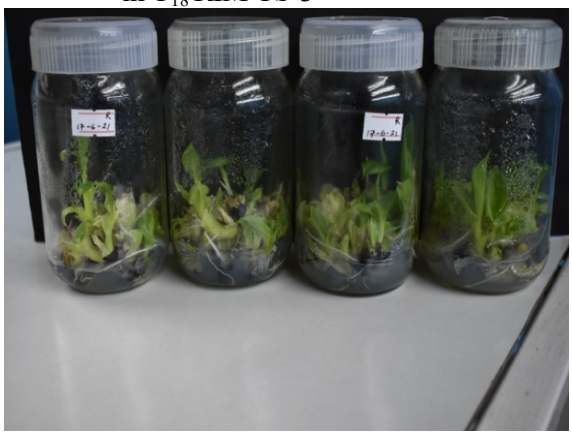


Initiation of shoots for rooting in T_{17} RIM TS-2



Initiation of shoots for rooting in T_{18} RIM TS-3

Rooting in T_{17} RIM TS-2



Rooting in T_{17} RIM TS-2

Fully rooted plantlets ready for acclamatization

Plate 20 Effect of Table sugar 30 g/l in rooting of banana explants (MFBs) cv Chinichampa (AAB)

4.5 Experiment – 5

4.5.1 Sub culture studies on *Musa* cv. Chinichampa (AAB)

Sub culturing is the process of removing plant cells from their older media and transferring them to a fresh medium. This enables further proliferation of the cells. The plant tissues are cleaned and divided into small sections and then transferred to fresh medium. The number of times the sub culturing is done is known as sub culture cycle. In this study the media showing the best result for multiple bud regeneration and rooting was taken forward for sub culturing studies up to the fifth cycle. The sub culturing was repeated at four weeks interval.

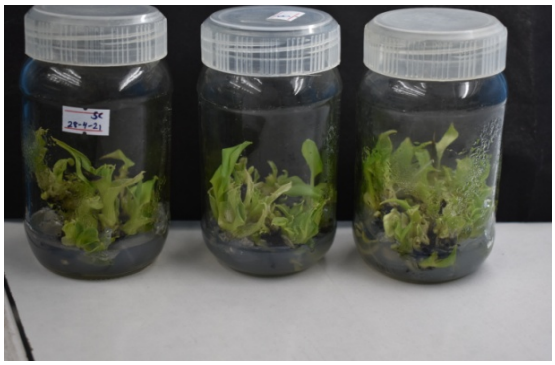
In the study the medium containing 30 g table sugar (T_{18}) recorded the best response in regeneration of multiple buds in both the explants under study and therefore the sub culture studies were recorded in this medium. Significant increased in the number of multiple buds per explant was observed up to third subculture and then showed slight decreased in both the explants under study. 100% response was observed in first and third sub culture in both the explants studied. Similar finding were observed by Jambhale *et al*. (2001) who noticed that the average number of multiple shoots per explants decreases with the increase in subculture. However Lima *et al*. (2006) reported bacterial contamination, results in reduced rate of multiplication and number of subculture. The regeneration of multiple buds from shoot tip and MFBs segments in each sub culture of *Musa* cv. Chinichampa (AAB) was recorded and presented in Table 4.5.

Table 4.5 Subculture studies on regeneration of multiple buds from shoot tip and MFBs segments of *Musa* cv. Chinichampa (AAB)

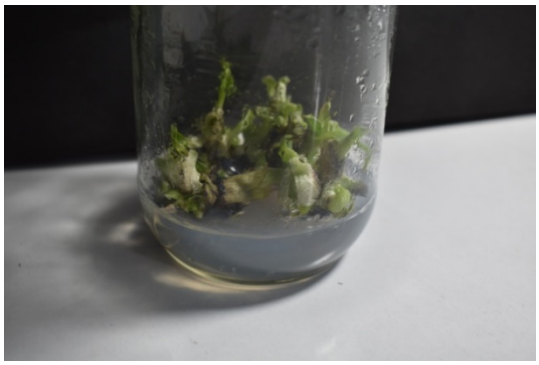
Subculture	Micro shoots responded for shoot production *in vitro* (%)		After 4 weeks	
			Number of multiple buds	
	Shoot tips	MFBs	Shoot tips	MFBs
1st sub culture	40(100.00)	40(100.00)	3.17	8.28[ab]
2nd sub culture	36(90.00)	36(90.00)	3.34	8.41[a]
3rd sub culture	40(100.00)	40(100.00)	3.71	8.92[a]
4th sub culture	36(90.00)	36(90.00)	3.38	8.36[a]
5th sub culture	36(90.00)	36(90.00)	3.14	7.19[a]
C.D. (5%)			NS	1.07
SE (m)			0.44	0.34

*Number of micro shoots inoculated in each sub culture was 40

*Means with different superscript letters within column are significantly different from each other by DMRT (*p=0.05*)



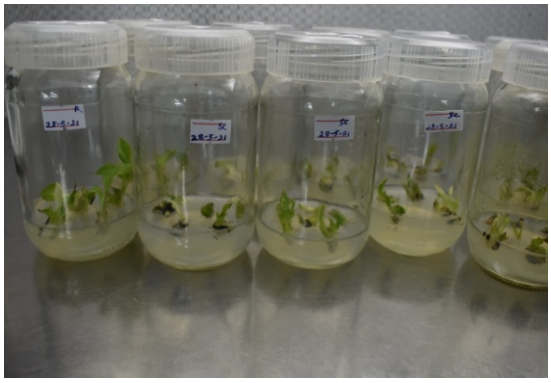
A) Subculture in T_{18} SPM TS-3 medium through shoot tip



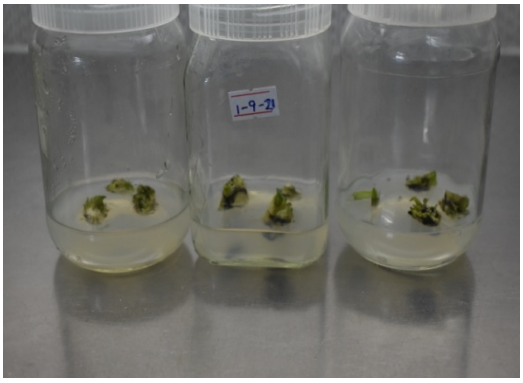
B) Subculture in T_{18} SPM-TS medium through MFBs

C) Excision of multiple buds in laminar flow from shoot tip culture

D) Excision of multiple buds in laminar flow from MFBs culture



E) Innoculation of excised buds from shoot tips in culture medium



F) Innoculation of excised buds from MFBs in culture medium

Plate 21 Different stages of *in vitro* subculturing on regeneration of multiple buds from shoot tip and MFBs segments of *Musa* cv. Chinichampa (AAB)

4.6 Experiment – 6

4.6.1 Hardening

In vitro rooted plantlets were hardened sequentially after successful plantlets development. Rooted plantlets were first taken out from culture bottles. Then thoroughly removed all the traces of media in running tap water, followed by soaking the root parts in Bavistin solution (0.1%) for an hour. Thereafter soaked in activated charcoal solution for 10 minutes before transferring to primary potting media.

Primary hardening is done in small plastic pots containing different media composition *viz*., Cocopeat alone; Cocopeat : Perlite (1:1); Cocopeat : Soil : Perlite (1:1:1). It was recorded that Cocopeat : Perlite (1:1) medium took the minimum days (24) with 90% plantlets survived. Whereas medium composition of Cocopeat : Soil : Perlite (1:1:1) took the maximum days (30) with 75 % plantlets survived (Table 4.6)

Secondary hardening was trialed in a soil mixture composition of Sand: Soil : FYM (1:1:1). It recorded a survival percentage of 98% in 60 days. The number of plantlets trialled under each treatment was 50 (Table 4.7).

Table 4.6 Mean survival percentage of *in vitro* plantlets during Primary hardening of *Musa* cv. Chinichampa (AAB)

Media Composition	Duration taken (days)	% Survival
Coco pith alone	28	80
Coco pith : Perlite (1:1)	24	90
Coco pith: Soil : Perlite (1:1:1)	30	75

*No. of plantlets trialed under each treatment = 50

Table 4.7 Mean survival percentage of *in vitro* plantlets during Secondary hardening of *Musa* cv. Chinichampa (AAB).

Media composition	Duration taken for Secondary Hardening (days)	% Survival
Sand: Soil:FYM (1:1:1)	60	98

*No. of plantlets trialed under each treatment = 50

A) Primary hardening of plantlets in Cocopeat

B) Primary hardening of plantlets in Cocopeat : Perlite (1:1)

C) Primary hardening in Cocopeat: Soil : Perlite (1:1:1)

D) Secondary hardening of plantlets in Sand:Soil:FYM (1:1:1)

Plate 22 Different stages of primary and secondary hardening of *in vitro* rooted plantlets of *Musa* cv. Chinichampa (AAB)

4.7 Experiment – 7

4.7.1 Differential cost analysis

In the present study, different carbon sources *(i.e.* sucrose, glucose, fructose, mannitol, sorbitol, maltose, table sugar and jaggery) was experimented and the differential cost of production for 1000 plantlets by different carbon sources was worked out as below in Table 4.8. In the present study it can be observed that using table sugar 20 g per litre as carbon source was found to be cheaper alternative to other carbon sources for shoot proliferation and rooting of Musa cv. Chinichampa (AAB). However, maltose 40 g per litre showed highest cost of production of 1000 plantlets in the present experiments. Bananas can be cultured on different carbon sources however table sugar showed maximum number of shoots per explants and this might be attributed to the presence of 96-97% sucrose in table sugar, compared to 99.98% in laboratory – grade sucrose Tyagi *et al*. (2007). There was 23.73 % cost reduction by replacing laboratory – grade sucrose with commercially available table sugar in the present study.

Table 4.8 Differential cost analysis for production for 1000 plantlets with different carbon sources

Carbon sources	Quantity / l (g)	Price/ 500 g (Rs)	Price / l (Rs)	Price/ 1000 plantlets (Rs) @ 8 L media	Price of 500g Agar (Rs)	Price of Agar for 8l media @7g / l (56g) (Rs)	Total of CS & Agar (Rs) (90% of Media cost)	Remaining 10% of media cost (Rs)	100% Media cost (or 20% of total cost of production) (Rs)	80% cost of production (Electricity & Labour cost) (Rs)	100%cost of production of 1000 plantlets (Rs)	Per unit cost of plantlets (Rs)	Add Pri & Sec Hardening cost @ Rs. 6 per net pot/ plantlets
Sucrose	30	340	20.39	163.12	4142	463.91	627.03	69.67	696.7	2786.8	3483.5	3.48	9.48
G-2	20	407	16.28	130.24	4142	463.91	594.15	66.02	660.17	2640.68	3300.8	3.30	9.30
G-3	30	407	24.42	195.36	4142	463.91	659.27	73.25	732.52	2930.08	3662.33	3.66	9.66
G-4	40	407	32.56	260.64	4142	463.91	724.55	80.51	805.06	3220.24	4025.3	4.03	10.03
F-2	20	867	34.68	277.44	4142	463.91	691.35	76.82	768.17	3072.68	3840.85	3.84	9.84
F-3	30	867	52.00	416.00	4142	463.91	879.91	97.77	977.68	3910.72	4888.4	4.88	10.88
F-4	40	867	69.36	554.88	4142	463.91	1018.79	113.20	1131.99	4527.96	5659.95	5.66	11.66
Man-2	20	1446	57.84	462.72	4142	463.91	926.63	102.96	1029.59	4118.36	5147.95	5.15	11.15
Man-3	30	1446	86.74	693.92	4142	463.91	1157.83	128.65	1286.48	5145.92	6432.4	6.43	12.43
Man-4	40	1446	115.68	925.44	4142	463.91	1389.35	154.37	1543.72	6174.88	7718.6	7.72	13.72
Mal-2	20	1375	55.00	440.00	4142	463.91	903.91	100.44	1004.35	4017.40	5021.75	5.02	11.02
Mal-3	30	1375	82.48	659.84	4142	463.91	1123.75	124.86	1248.61	4994.44	6243.05	6.24	12.24
Mal-4	40	1375	110.00	880.00	4142	463.91	1343.91	149.33	1493.24	5972.96	7466.2	7.47	13.47
Sor-2	20	1162	46.48	371.84	4142	463.91	835.75	92.86	928.61	3714.44	4643.05	4.64	10.64
Sor-3	30	1162	69.70	557.60	4142	463.91	1021.51	113.51	1135.02	4540.08	5675.1	5.67	11.67
Sor-4	40	1162	92.96	743.68	4142	463.91	1207.59	134.18	1341.77	5367.08	6708.85	6.71	12.71
TS-2	20	30	1.20	9.60	4142	463.91	473.51	52.62	526.13	2104.52	2630.65	2.63	8.63
TS-3	30	30	1.79	14.32	4142	463.91	478.23	53.14	531.37	2125.48	2656.85	2.66	8.66
TS-4	40	30	2.4	19.20	4142	463.91	483.11	53.68	536.79	2147.16	2683.95	2.68	8.68
J-2	20	100	4	32.00	4142	463.91	495.91	55.11	551.02	2204.08	2755.1	2.76	8.76
J-3	30	100	5.99	47.92	4142	463.91	511.83	56.87	568.70	2274.80	2843.5	2.84	8.84
J-4	40	100	8	64.00	4142	463.91	527.91	58.66	586.57	2346.28	2932.85	2.93	8.93

CHAPTER V

SUMMARY AND CONCLUSION

SUMMARY AND CONCLUSION

The present investigation entitled “Responses of various carbon sources for *in vitro* regeneration of banana cv. Chinichampa (AAB)” was carried out at Department of Horticulture, at School of Agricultural Sciences, Medziphema, Nagaland University during the year 2019-2022. The experiment was conducted in Completely Randomized Design (CRD) with twenty two treatments in three replications to estimate the responses of various combinations of carbon sources on *in vitro* culture establishment, shoot proliferation, rooting and hardening and differential cost analysis. The results thus obtained are summarized below:

- In experiment – 1, amongst the various media treatments trialed, T_{18} SPM TS-3 (table sugar 30 g/l) showed the highest number of multiple buds produced per explant at 4.00. However the length of multiple buds was found highest in T_1 SPM S (sucrose 30 g/l) at 3.03 cm from shoot tip culture.

- In experiment – 2, amongst the various media treatments trialed through immature male floral buds, T_{18} SPM TS-3 (table sugar 30 g/l) produced the highest number of multiple buds per explant at 8.33 which was at par with T_1 SPM S (sucrose 30 g/l). However length of multiple buds was recorded highest under T_1 SPM S (sucrose 30 g/l) at 3.01 cm.

- In experiment – 3, amongst the various media treatments trialed, T_{18} RIM TS-3 (table sugar 30 g/l) resulted in highest number of roots per shoot (4.59). Meanwhile T_{17} RIM TS-2 (table sugar 20 g/l) recorded the longest roots (7.74 cm) from shoot tip culture.

- In experiment – 4, amongst the various treatments trialed, T_1 RIM S (sucrose 30 g/l) showed the highest number of roots per shoot at 4.87.

Meanwhile T_{18} RIM TS-3 (table sugar 30 g/l) recorded the longest roots (6.68 cm) from immature male floral buds culture.

- Subculturing was successfully repeated at every 4 weeks interval up to 5^{th} subculture and then maximum number of multiple buds (3.71 and 8.92) were recorded during 3^{rd} subculture in T_{18} SPM TS-3 (table sugar 30 g/l) in both the explants under experimentation.

- Mean survival percentage of *in vitro* plantlets during primary hardening was highest at 90% under coco peat:perlite (1:1) combination. While the mean survival percentage during secondary hardening was observed at 98% under sand:soil:FYM (1:1:1).

- Differential cost analysis for production of 1000 plantlets with different carbon sources was found lowest under Table sugar 20 gm/l at Rs. 2630.65 (Rs. 8.63/hardened plantlet). There was 23.73 % cost reduction by replacing laboratory – grade sucrose with commercially available table sugar in the present study.

Through these studies we can suggest that more research works should be carried out on different cheaper carbon sources, standardization of sterilization techniques, different cheaper gelling substances, different plant growth regulators, size and choice of explants, etc. Researchers should always keep in mind on alternative cheaper and readily available substitutes to reduce the plantlet cost acceptable for commercial adoption by tissue culture industry. It is suggested to do more experiments on combinations of carbon sources and growth hormones along with other additives for better understanding the physiology and morphogenesis of *in vitro* regeneration. Molecular studies using ISSR markers should be employed to ascertain the true to type of the elite cultivar to avoid chromosomal aberrations. Lastly, more research should be carried out using immature male inflorescence because of its absence of lateral contaminations and also to ease with collection of large no. of suckers for shoot

tips culture. Also inflorescence culture showed promising results in multiplication ratio of *in vitro* plantlet regeneration.

Thus, from the present investigation on the "Responses of various carbon sources for *in vitro* regeneration of banana cv. Chinichampa (AAB)", it is concluded that Table sugar 30 g/l as carbon source was best for *in vitro* shoot establishment, proliferation and rooting of banana cv Chinichampa (AAB) for both the explants under study. While subculture can be successfully obtained up to 5^{th} subculture without any contaminations. Primary hardening can be best achieved in cocopeat : perlite (1:1), while secondary hardening can be successfully achieved in sand : soil : FYM (1:1:1) combination. Therefore, it can be concluded that table sugar can be the best alternate carbon source for *in vitro* propagation of banana cv Chinichampa (AAB) as compare to other high cost laboratory - grade carbon sources.

CHAPTER VI

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