PROPAGATION STUDIES ON CLONAL ROOTSTOCKS OF APPLE (*Malus* × *domestica* Borkh.)

by

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Ph.D

PROPAGATION STUDIES ON CLONAL ROOTSTOCKS OF APPLE (*Malus* × *domestica* Borkh.)

Manmohan Lal

2021

CERTIFICATE - I

This is to certify that the thesis entitled **"Propagation Studies on Clonal Rootstocks of apple (*Malus* × *domestica* Borkh.)"** submitted in partial fulfilment of the requirements for the degree of **Doctor of Philosophy in Horticulture (Fruit Science)** to the Faculty of **Agriculture**, Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu, is original work and has similarities with published work not more than minor similarities as per UGC norms of 2018 adopted by the University. Further, the level of minor similarities has been declared after checking the manuscript with **Urkund** software provided by the University.

The work has been carried out by **Mr. Manmohan Lal** under my supervision and guidance. No part of the thesis has been submitted for any other degree of diploma. It is further certified that help and assistance received during the course of thesis investigation have been duly acknowledged.

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ABSTRACT

Title of the Thesis	:	Propagation Studies on Clonal Rootstocks of Apple (*Malus* × *domestica* Borkh.)
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ABSTRACT

The present investigations entitled "Propagation studies on clonal rootstocks of apple (*Malus* × *domestica* Borkh.)" was carried out at Plant Tissue Culture Laboratory, School of Biotechnology (SBT) and Tissue Culture Laboratory, Advanced Centre for Horticulture Research (ACHR), Udheywalla of Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu to standardize the *in vitro* propagation protocol of clonal apple rootstocks MM106 and MM111 during 2018. The shoot tip and nodal segment used as explants were taken from MM106 and MM111 mother block maintained at Regional Horticulture Research Sub Station, Bhaderwah, SKUAST-Jammu. Results from the present investigation revealed that different treatments of mercuric chloride ($HgCl_2$) and sodium hypochlorite (NaOCl) showed a significant effect on per cent aseptic and per cent survival of cultures. Treatment of 0.1 per cent $HgCl_2$ for 4 minutes resulted in maximum aseptic and survival of cultures during all the months of study. MS medium supplemented with 10 mg/l polyvinyl pyrrolidone was found to be the best antioxidant medium for reducing phenolic exudation from the excised portion of the explants and for culture establishment of explants. Nodal segments of clonal apple rootstock MM106 and MM111 resulted in maximum percentage of culture establishment i.e. 32.22 per cent and 47.88 per cent, respectively as compared to shoot tip explants. MS medium supplemented with 1.0 mg/l BAP + 1.0 mg/l GA_3 was found to be the best medium for culture establishment in minimum number of days. Nodal segments were found to be more suitable explants than shoot tips which resulted in maximum culture establishment percentage of 57.77 per cent and 76.66 per cent in 26.33 days and 25.40 days for rootstocks MM106 and MM111, respectively. However, different media combinations also showed a significant influence on *in vitro* shoot proliferation. Nodal segments of clonal apple rootstocks MM106 and MM111 were found to be better in comparison to shoot tip explants wherein minimum number of days taken for shoot proliferation were recorded as 46.26 and 44.70, highest number of shoots per explant as 4.76 and 5.43, length of the longest shoot as 3.42 cm and 3.47 cm and highest number of leaves per explant as 10.33 and 11.00, respectively on MS medium supplemented with 1.0 mg/l BAP + 1.0 mg/l GA_3 along with 0.2 per cent NAA.

Moreover, different media compositions showed a significant effect on *in vitro* rooting of clonal apple rootstocks. The clonal apple rootstock MM111 was found to be superior for *in vitro* rooting response as compared to MM106 rootstock. Half strength MS medium supplemented with 1.0 mg/l NAA along with 0.2 per cent activated charcoal resulted in maximum rooted cultures as 60.00 per cent and 66.67 per cent and took minimum number of days of root initiation as 14.00 and 13.00 in clonal apple rootstocks MM106 and MM111, respectively. Maximum number of roots per explant as 5.00 and 5.67 and maximum root length as 3.70 cm and 3.75 cm in clonal apple rootstock MM106 and MM111, respectively were also recorded with half strength MS medium supplemented with 1.0 mg/l NAA along with 0.2 per cent activated charcoal.

The potting mixture containing soil: sand: vermiculite: FYM :: 1:1:1:1 was found to be the most appropriate for the growth of *in vitro* raised plantlets of clonal apple rootstocks MM106 and MM111 which resulted in maximum survival of 73.33 per cent and 76.67 per cent, plant height as 3.23 cm and 3.30 cm and number of leaves/ plantlets as 9.00 and 11.00, respectively. The unit cost of one *in vitro* raised plantlets of clonal apple rootstocks i.e. MM106 and MM111 in present investigation was estimated to be ₹ 44.56 and ₹ 36.95, respectively including both tissue culture production phase and greenhouse production phase.

Keywords: Clonal rootstock, propagation, *in vitro*, apple, economics, shoot tip, nodal segment

Signature of the Advisor **Signature of the Student**

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ABBREVIATIONS

%	:	Per cent
MT	:	Metric tonnes
et al.	:	Et alia, and others
i.e.	:	*Id est* (that is)
^{o}C	:	Degree Celsius
ml	:	Millilitre
mg	:	Milligram
viz.,	:	Vide licet, namely
FYM	:	Farm yard manure
J&K	:	Jammu and Kashmir
CD	:	Critical difference
2,4-D	:	2,4- Dichlorophenoxyacetic acid
BAP	:	6-Benzylaminopurine
HCl	:	Hydrochloric acid
$HgCl_2$	:	Mercuric chloride
NaOCl	:	Sodium hypochlorite
IAA	:	Indole-3-acetic acid
IBA	:	Indole-3-butyric acid
GA_3	:	Gibberellic acid
NAA	:	α-naphthalene acetic acid
AC	:	Activated charcoal
ppm	:	Parts per million
EDTA	:	Ethylenediaminetetraacetic acid
µM	:	Micro molar
pH	:	Negative logarithm of hydrogen ion concentration
v/v	:	Volume/ volume
N	:	Normal solution
TDZ	:	Thdiazuron
/	:	Per
no.	:	Number
NaOH	:	Sodium hydroxide
cm	:	Centimeter
l	:	Litre
MS	:	Murashige and Skoog

WPM	:	Woody Plant Medium
cv.	:	Cultivar
g	:	Gram
PVP	:	Polyvinyl pyrrolidone
ha	:	Hectare
USAID	:	United States Agency for International Development
MM	:	Malling Merton
EMLA	:	East Malling and Long Ashton
G	:	Geneva series
B	:	Budagovsky series
DICA	:	Sodium dichloroisocyanurate
$CaOCl_2$	:	Calcium hypochlorite
KN	:	Kinetin
ip	:	Isopropanol
@	:	At the rate
Fe-EDDHA	:	Ethylenediamine di-2-hydroxyphenyl acetate ferric
PG	:	Phoroglucinol
½	:	Half
sp.	:	Species
FYM	:	Farmyard manure
₹	:	Rupees
SMD	:	Star Micronics Devices
SDW	:	Sterile Distilled Water
Lux	:	SI derived unit of illuminance
CRD	:	Completely Randomized Design
BAR	:	6 - Benzyl aminopurine riboside
etc.	:	Etcetera
TOP	:	Meta-topolin
N	:	Normal
µg	:	Microgram
SKUAST-Jammu	:	Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu

Chapter-I

Introduction

CHAPTER-I

INTRODUCTION

The cultivated apple (*Malus* × *domestica* Borkh.) has originated from South-Western Asia, in the Caucasus region near Gilan in Kazakhstan. The genus *Malus* belongs to sub family Pomoideae and family Rosaceae with basic chromosome number 17 (Brown, 1992). The genus *Malus* has 25 to 30 species and several sub-species of the so called crab apples, many of which are cultivated as ornamental trees for their profuse flowers and attractive fruits (Way *et al*., 1990 and Li, 1996). Most of the species of apple are intercrossed because self-incompatibility is common in the genus so the plants produced from seed are mostly inter-specific hybrids. Hence, the cultivated apple is likely the result of interspecific hybridization and at present the binomial *Malus* × *domestica* has been generally accepted as the appropriate scientific name (Korban and Skirvin, 1994).

Morphologically, apple is a deciduous, perennial tree, 5-12 meter tall and with a longevity between 60 to 100 years. The leaves are alternately arranged, simple oval with an acute tip and serrated margin, 5-12 cm long and 3-6 cm broad which are attached on a 2-5 cm petiole (Dobrzanski *et al*., 2006). Typical flower of apple are white to pink in color, consists of five petals, calyx of five sepals, about twenty stamens and the pistil which divides into five styles. The ovary (fruit) has five carpels at the center of the fruit which are arranged in a five point star, each usually containing one to three ovules (Janick *et al*., 1996). The fruit is pome (developed from inferior ovary), usually without grit-cells, persistent or deciduous calyx, with depression at either end. The edible portion of fruit is fleshy thalamus. Most of the apple varieties requires 1000-1500 chilling hours below 7 ^{o}C to break the rest period. These conditions are available at an altitude ranging from 1500-2700 m above mean sea level. The optimum temperature should be between 21.1-26.7 ^{o}C for pollen germination and fruit setting whereas average optimum summer temperature should be around 21-24 ^{o}C during the active growing season. In general, sites located on eastern aspect at lower altitudes are suitable for optimum apple growth. Apple trees are particularly sensitive to low moisture supply, so for an optimum growth and fruitfulness, apple trees require 100-125 mm of rainfall equally distributed over the growing season. It can be grown in all types of soils except heavy clay and compact

sub soil. Proper drainage and good fertility of soil are important. Loamy soils, rich in organic matter content with pH ranging from 5.5-6.5 having good aeration are reported to be ideal for proper growth and development in apples. China, Russia, India, Ukraine, Poland, USA, Iran, Turkey, Korea, Germany, Italy, Argentina, Japan, Spain, Chile, Brazil etc. are the major apple producing countries of the world. At present, in India, apple is cultivated over an area of about 314000 ha producing 2503000 MT of the fruit (Anonymous, 2019a). In Jammu and Kashmir, apple fruit covers 164742 ha area with an annual production of 1882319 MT, whereas, Jammu province produces 30595 MT of apple from an area of 18415 ha (Anonymous, 2019b).

Apple has a large popularity among the consumers both as a table fruit and value added processed products in India as it is rich source of carbohydrates, proteins, minerals *viz.*, calcium (6.0 mg), phosphorous (11.0 mg/100g), iron (0.12 mg/100g), magnesium (5.0 mg/100g), potassium (107 mg/100g) and sugars, thiamine (0.017 mg/100g) and vitamin B_6 (0.041 mg/100g) (Bal, 2014 and Skinner *et al*., 2018). Apple is believed to reduce the incidence of dental cavities, helps to control obesity and supply extra energy for heavy exercise. The proverb “an apple a day keeps the doctor away” strengthens the idea that apple fruit is an excellent source of very important food nutrients. It is chiefly used in preparation of products like jam, jelly, preserves, slices, sauces, apple butter, apple chips, apple rings, wine, juice, concentrated juice, cider, and powder (Chaudhary *et al*., 2014).

Haberlandt (1902) revealed that for every million cells that make up the plant body, it has the ability to reproduce and is predetermined to reproduce and reproduce itself in developmental events that occur after the formation of the zygote, a substance called totipotency. Jones (1967) first introduced apple to micropropagation when it was discovered that the growth regulator, benzyl amino purine (BAP), could stimulate the growth of a shoot tip. Since then a large number of apple cultivars and rootstocks has been successfully propagated through tissue culture. In Jammu province of the J&K, rootstocks of apple are mainly raised from seeds. The seedling plants are more vigorous and show variability in their performance. Depending on the growth behavior, the clonal rootstocks of apple are divided into three major groups including strong growing (standard), medium-strong growing (semi-dwarf) and weak growing (dwarf) types. Now-a-day’s many clonal rootstocks (M9, M4, M7, M27,

MM106, MM111, MM104, Merton793, G814, G41, G213, 814, G935, G222, G969, G30, G210, G890, Ottawa3, B9, B18, B10, V1, EMLA7, EMLA26, P18 etc.) have been developed for apple which is grown in wide ecological range, precocious, true to type, resistance to various diseases, insect pests, viruses and adaptable to different climate and soil conditions. Thus, they are considered better than seedling rootstocks. Moreover, clonal rootstocks have tree size control over the scion variety. Apple clonal rootstocks are generally propagated through mound layering or trench layering. These conventional methods are totally dependent on season, take longer time, has lower multiplication rates and require large land area for plantlet production and the resultant planting material produced may be exposed to soil borne pathogens, attacked by insect pests and viruses that leads to degradation in quality of planting material.

Over the last two decades, several research groups purposed different *in vitro* conditions that were suitable for micropropagation of apple cultivars and rootstock material (Noiton *et al*., 1992, Skirvin *et al*., 1986, Marin *et al*., 1983 and Quoirin and Lepoivre, 1977). Rapid plant propagation through tissue culture technique called micropropagation can be achieved in a short time and space. Thus, it is possible to produce plants in large number and with lower costs starting from single individual tissue (Razdan, 1993). Future projections for the apple industry also indicate that demand for improved plant material will continue to grow as new ones continue to replace older varieties and rootstocks. *In vitro* propagation technique is also reported to be useful for the production of stock plants with improved root quality (Quamme and Hogue, 1994 and Webster and Jones, 1991).

Considering the soil and climatic conditions and the topography of apple growing areas in Jammu division, the clonal rootstocks of apple MM106 and MM111 are high in demand. MM106 is a cross of Northern Spy × M1 and most popular rootstock in its category. It is a semi dwarf, precocious rootstock and slightly larger than Malling 7. It was released from East Malling Research Station, England. An apple tree propagated on this rootstock is well anchored and starts bearing fruit in 3-4 years. It grows well and reaches a final height up to 10-15 feet depending upon the cultivar used. It has tree size of 80 per cent on non - spur type and 70 per cent on spur type. It is resistant to wooly apple aphid, burr knot and ideal for high density planting. MM111 is introduced in the year 1952 from a cross of Merton793 × Northern Spy by

John Innes Horticultural Institute and East Malling Research Station, England. It is semi vigorous, producing a tree size about 85-90 per cent of the size of the tree on seedling rootstock. It is resistant to wooly apple aphid and quite tolerant to fire blight and crown and root rots. It is fairly winter hardy and produces moderate amount of burr knot and suckers. Since, the demand of these clonal rootstocks of apple is very high in temperate areas of Jammu region and the availability is very less and considering the importance of these rootstocks as pointed above, our present study entitled "Propagation studies on clonal rootstocks of apple (*Malus* × *domestica* Borkh.)" was carried out with the following objectives:

1. To standardize the protocol for *in vitro* propagation of apple clonal rootstocks MM106 and MM111.
2. To find out the economics of *in vitro* propagation of apple clonal rootstocks MM106 and MM111.

Chapter-II

Review of Literature

CHAPTER - II

REVIEW OF LITERATURE

The tissue culture of domesticated apple (*Malus* × *domestica* Borkh.) has a rich and extensive history spanning approximately 60 years (Dobranszki and Silva, 2010). *In vitro* culture has its best commercial application on production of clonal plants at a very rapid rate as compared to conventional methods of propagation. It has also proved to be of immense practical value as an aid to the production and maintenance of disease free plants, germplasm conservation and a valuable adjunct to the conventional methods of plant improvement including plant genetic engineering. It is said that the application and development of tissue culture technique are opening the doors to a second green revolution. Thus, literature pertaining to the study entitled "Propagation studies on clonal apple rootstocks of apple (*Malus* × *domestica* Borkh.)" has been reviewed as under:

2.1 General review on *in vitro* techniques

Murashige (1974) described the regeneration of plant, accomplished by explants from shoot tips, leaves, stem, cotyledons and microsporocytes. The most important determinant of plant multiplication and quality of regenerated plants is the initial explant (Gamborg *et al*., 1974). Plant tissue culture mainly for regeneration of elite and rare species is an important alternative to the conventional method of vegetative propagation (Rao *et al*., 1996). Plant tissue culture is the best technique for production of large scale propagules especially in endangered medicinally plant species where explant material is available in a very less amount.

Micropropagation of mature trees with vegetative explants has been a difficult task due to various factors i.e. presence of phenolic compounds, exogenous and endogenous infection, maturity, juvenility, slow growing habit, long genetic variations (Bajaj, 1991, Bonga and Durzan, 1986, Zimmerman, 1985 and Durzan, 1984). The micropropagation technique has been used for the rapid and large-scale propagation of some fruit and forest trees (Hutchinson and Zimmerman, 1987). Tissue culture techniques are used for somatic hybridization, genetic manipulation and virus eradication.

First report of apple tissue culture was given by Letham (1958). Various advantages of the technique over traditional practices have been discussed by a Zimmerman (1984). Extensive work started on shoot tips of M7 and M26 rootstocks of apple by Jones *et al*. (1985) who suggested that 60,000 green shoots can be produced from the single shoot apex in eight months. Plant tissue culture was revolutionized with the development of a defined culture medium by Murashige and Skoog (1962). Plant Tissue Culture Technology is being used for large-scale production of elite planting material of desired characteristics. *In vitro* methods can be used to produce, maintain, multiply and transport pathogen free plants safely and economically. Through plant tissue culture, any plant part can be cultured on a nutrient medium under aseptic conditions. At present MS medium is the most effective and widely used culture medium for various plant species.

Presently, plant tissue culture technique has gained unbeatable recognition in plant science for successful micropropagation and improvement of plant species, leading to its commercial application. Tissue culture is a logical method for the production of self-rooted clonal apple trees as it has the potential to produce a large number of plants in less time (Bhatt *et al*., 2012).

2.2 Surface sterilization and type of explants

Surface sterilization is a very important and basic aspect of plant tissue culture, as it actually aims at *in vitro* propagation of progenies of desired genotypes free from surface and systemic contamination. There are wide range of factors which need to be consider when disinfecting the field or *ex vitro* derived plant material to be used as explants for *in vitro* culture, but the most important factors are genotype, explant type, disinfection procedure, age and physiological state of the mother plant and physical and chemical conditions applied *in vitro* (Silva *et al*. 2016). Any plant part, organ or tissue can be used as explants. Actively growing shoot tips are used as meristem, shoot tip, and bud cultures because of their growth potential and low virus load (Ault, 1994).

Moore (1986) reported that sterilization of internodal stem sections of citrus rootstocks with 1N HCL for 5 minutes significantly reduces contamination percentage. The single node explants of chinese gooseberry established well when treated with $HgCl_2$ for 3-8 minutes (Shen *et al*., 1990).

Shoot tips of *Citrus aurantifolia* were disinfected with 1.0 per cent NaOCl followed by 1.0 per cent $HgCl_2$ for different time intervals and optimum percentage of aseptic cultures were observed with 10 minutes NaOCl treatment followed by 5 minutes $HgCl_2$ (Rana and Singh, 2002). Mereti *et al*. (2002) obtained a success of the sterilization of the strawberry explants by steeping explants in a sodium hypochlorite solution (1.5%) for 10 minutes.

Singh and Pandey (2004) obtained contaminated free explants of Pajaro and Sweet Charlie strawberry by surface sterilization with sodium hypochlorite for 10 minutes followed by washing with sterile distilled water.

Kaushal *et al*. (2005) reported minimum browning intensity and 60 per cent survival rate after six weeks of cultures in MM111 apple rootstock using shoot tip of size 0.2 to 0.4 cm and 0.4 to 0.6 cm.

Kour *et al*. (2005) treated vegetative buds of Chandler strawberry plants with 0.5 per cent carbendazin and surface sterilized with solution of 0.1 per cent $HgCl_2$ for 2-3 minutes and proved that the treatment provided very less contaminated vegetative buds percentage.

Sakila *et al*. (2007) reported that when nodal segments of strawberry are washed in running water and washed again by adding Tween-20 and finally surface sterilized with mercuric chloride in laminar flow chamber for 5 minutes produced maximum percentage of uncontaminated buds.

Axillary buds (0.5-2.0 cm) of Merton 793 apple rootstock when surface sterilized with mercuric chloride ($HgCl_2$) for 4 minutes provided highest number of uncontaminated buds (90.00%), but the survival rate was more in exposure time of 3 minutes (Soni *et al*., 2010).

Boudabous *et al*. (2010) reported that axillary buds of Douce de Djerba apple plants when surface sterilized with calcium hypochlorite ($CaOCl_2$) at 5.0 g/l for 5 minutes provided maximum uncontaminated percentage (96%).

Nodal segments (0.3-0.8 cm) showed minimum contamination rate (48.1% and 88.3%) in Fereley Jaspi and Giesla pear rootstock, respectively after surface sterilization with 70 per cent ethanol for 1 minute and 20 seconds followed by soaking in bleach solution at 0.4 per cent for 12 minute (Vujovic *et al*., 2012).

Jan *et al*. (2013) reported that maximum aseptic cultures (80.00% and 76.66%) in strawberry cv. Senga Sengana were recorded from both explants *viz.*, runner tips and nodal segments, respectively when treated with 1.5 per cent NaOCl for 20 minutes plus ethyl alcohol 70 per cent for 30 seconds, but the surviving percentage was less because this treatment resulted in necrosis and tissue injury of explants. However, 0.1 per cent $HgCl_2$ for 4 minutes resulted in less aseptic cultures percentage but gave highest survival percentage of explants.

Mihaljevic *et al*. (2013) carried out a study with an aim to establish best surface sterilization for *in vitro* propagation of Oblacinska sour cherry plants. Results from an experimental study indicated that among all surface sterilizing agents, silver nitrate ($AgNO_3$) at concentration of 1.0 per cent for 20 minutes was found to be best for controlling the infection, whereas, sterilization with 1 per cent sodium dichloroisocyanurate for 10 minutes was not found satisfactory.

The highest survival response of 31.7 per cent was showed by 0.05 per cent $HgCl_2$ treatment followed by 4 per cent $CaOCl_2$ (29.2% and 26.7%) treatments for 5 and 10 minutes, respectively in case of nodal explants. Further, the maximum survival response (48.30%) was observed when shoot tips of guava plants were exposed to 2 per cent NaOCl for 5 minutes (Zamir and Rab, 2014).

Lohidas and Sujin (2015) carried out a study on effect of growth hormones in the micropropagation of banana cv. Matti and revealed that highest contamination free cultures were obtained in treatment with 95 per cent alcohol for 30 seconds followed by 1percent sodium hypochlorite for 10 minutes and 250 ppm streptomycin sulphate. Moreover, maximum survival (90.33 ± 0.58%) of explants after inoculation was obtained when the explants were sterilized with 95 per cent alcohol for 30 seconds followed by 1.0 per cent NaOCl for 10 minutes and 250 ppm streptomycin sulphate.

Axillary buds treatment with 2.0 per cent NaOCl for 3 minutes was found to be the best for surface sterilization that produced very less contamination (13.33%), followed by (25.00%) with 0.1 per cent $HgCl_2$ for 3 minutes in Gisela-5 cherry rootstock (Thakur *et al.*, 2016).

In an experiment was conducted on the factors affecting degree of browning and culture establishment in pomegranate with an aim to identify the most suitable

types and length of the nodal explants. Results revealed that axillary buds (1.5 cm) was found better for minimum browning and higher culture establishment percentage (Singh and Patel, 2016).

Lazo-Javalers *et al*. (2016) carried out a study on surface disinfection procedure and *in vitro* regeneration of grape vine cultivar Flame seedless. Results from the study indicated that best disinfection of 90 per cent tissue survival involved shaking for 60 minutes in a solution containing 20 per cent Clorox with an addition of 50 drops/l of Triton X-100 and these tissues showed the potential to regenerate into complete plants.

In a study, surface sterilization for reducing microbial contamination in *in vitro* propagation of Lasora (*Cordia myxa* Roxb.) using nodal segments, Padhi and Singh (2017) reported that explants treated with $HgCl_2$ (0.1%) for 10 minutes resulted in 85.50 per cent aseptic culture and 83.25 per cent survival of explants.

Lizarraga *et al*. (2017) carried out a study on *in vitro* propagation and recovery of eight apple and two pear cultivars held in a germplasm bank. Results from the study indicated that apical bud from axillary shoots of size 1.0-1.5 mm showed highest survival rate (50%) at establishment for both apple and pear cultivars.

During study on surface sterilization and *in vitro* propagation of *Prunus domestica* L. cv. Stanley using axillary buds as explants, Wolella (2017) reported that highest survival value (97%) were recorded in explants disinfected with 2 per cent NaOCl for 15 minutes and 0.1 per cent $HgCl_2$ for 7 minutes.

An experiment was carried out on optimization of surface sterilization and manipulation of *in vitro* conditions for reduced browning in pomegranate (*Punica granatum* L.) variety Bhagava. Results from the experiment revealed that explants exposed to 300 mg/l bavistin (50% carbendazim) for 18 minutes, 200 mg/l streptocycline and cefotaxime for 18 and 8 minutes respectively, followed by 1000 mg/l $HgCl_2$ gave 93.33 per cent axenic cultures with least contamination percentage of 6.67 per cent for fungal contaminants, among all the other treatment combinations (Patel *et al*., 2018).

Fatima *et al*. (2018) reported that maximum surface sterilization of walnut embryos was achieved by using 5 per cent NaOCl for 15 minutes, whereas surface sterilization of cotyledons was achieved in 5 per cent NaOCl for 25 minutes and for

other explants like shoot apices, leaves, apical and axillary buds, 5 per cent NaOCl for 25 minutes followed by 0.05 per cent $HgCl_2$ for 5 minutes was found effective.

In a study, Shukla *et al*. (2019) reported minimum contamination percentage (31.33%) in Udhayam banana explants after 10 days of treatment under 0.1 per cent $HgCl_2$ for 8 minutes while maximum (73.25%) was noted under treatment of 0.1 per cent $HgCl_2$ for a period of 2 minutes. They also reported that maximum survival percentage after 25 days (84.74%) was noted under treatment of 0.1 per cent $HgCl_2$ for a period of 2 minutes followed by 74.26 per cent and 53.18 per cent with the duration of 4 and 6 minutes, respectively.

2.3 *In vitro* culture establishment

A study of growth substances and photoperiod on shoot apices of *Vitis* cultured *in vitro* and their effects on sub cultured shoot tips revealed that addition of NAA (5×10^{-7}) along with BAP (5×10^{-6}) in the MS medium were found to be effective for establishment in Rougeon grape shoot culture (Chee and Pool, 1982).

Thidiazuron (0.45 to 2027 µM) alone or in combination with kinetin (0.5 or 1.0 µM) or BAP (0.5 or 1.0 µM) was recorded best for culture establishment in grape cv. Noble and Tarheel explants (Sundarsono and Goldy, 1988).

Singh *et al*. (2004) reported that MS medium augmented with 0.2 mg/l NAA and 4.0 mg/l BAP improved culture establishment percentage in grape cv. Pusa Urvashi.

Kaushal *et al*. (2005) reported that apple shoot tips of the size 0.2-0.4 cm and 0.4-0.6 cm showed less browning intensity and about 60 per cent of explants survived after six weeks of culture in MS medium fortified with1.0 mg/l BAP + 0.02 mg/l NAA+0.1 mg/lGA_3.

Kour and Singh (2012) conducted an experiment for rapid *in vitro* propagation of rough lemon from nodal segments to standardize the protocol for mass multiplication under laboratory conditions and reported that MS medium augmented with BAP (1.5 mg/l) + malt extract (500 mg/l) resulted in maximum culture establishment percentage (94.45%).

In an experiment studying factors affecting degree of browning and culture establishment in pomegranate, MS medium supplemented with 1.0 mg/l BAP + 0.5

mg/l NAA resulted in higher culture establishment percentage (68.5%) with explant of third node position (Singh and Patel, 2016).

Thakur *et al*. (2016) developed a technique for *in vitro* propagation of Gisela-5 cherry rootstock and reported that maximum *in vitro* establishment (70%) was achieved on MS medium supplemented with 0.5 mg/l BAP and 0.5 mg/l GA_3 during the month of July and February.

Lizarraga *et al*. (2017) carried out a study on eight apple and two pear rootstocks in order to define the culture establishment protocol. In this study, MS medium supplemented with 1.0 mg/l BAP, 0.2 mg/l GA_3 and 0.3 mg/l IBA was used and study revealed that the combined effect of BAP (1.0 mg/l) and GA_3(0.2 mg/l) had highest survival rate (55%) at establishment in apple cultivar Principe Grande.

2.4 *In-vitro* shoot proliferation

Good percentage of *in vitro* shoot proliferation rates is due to phenomenon of plant growth regulators. The plant growth regulators (BAP and KN) has been popularly considered as shoot proliferation hormones in plant tissue culture technique. Skoog and Miller (1957) reported for the first time that the ratio of auxin and cytokinin determine the type and extent of organogenesis in tissue culture. Dobranszki and Silva (2010) reported that choice of plant growth regulators is most imperative factor which governs the *in vitro* development and growth of apple shoots.

Pool and Powell (1975) studied the effect of various cytokinins on ‘Concord’ *in vitro* shoot cultures and reported that BAP at 1.0 mg/l or above induced shoot development as compared to zeatin riboside and 2–ip.

Shoot proliferation in *Vitis vinifera* cv. Sylvaner was achieved at 2×10^{-5} M, however, high BAP retard shoot elongation (Jona and Webb, 1978). Barlass and Skene (1980) also reported that medium supplemented with BAP (10^{-5} M) favors shoot regeneration in grape cv. Cabernet Sauvignon.

An optimum shoot proliferation rates were achieved in MS medium supplemented with cytokinins, BAP and zeatin both at 2.0 mg/l in Chenin Blanc grapes plant. (Goussard, 1981).

Harris and Stevenson (1982) reported maximum shoot production rates in MS medium supplemented with 80 mg/l adenine sulphate, 170 mg/l sodium phosphate and 3-4 mg/l BAP in grape French hybrid cv. Baco.

Pua *et al*. (1983) obtained normal growth of Ottawa 3 apple shoots when cultured on MS medium supplemented with (0.5 mg/l) NAA, (0.5 mg/l) BAP and (5.0 mg/l) GA_3. They also reported that maximum shoot weight (2.27 g) and length of the longest shoot (108.51 mm) was obtained when shoots were cultured in MS medium supplemented with NAA (0.5 mg/l) and BAP (0.5 mg/l). .

In a study, Gray and Klein (1989) noted that BAP (0.5-5 µM) in permutation with TDZ (5-20 µM) resulted in more number of shoots per shoot apex in cultivar Blanc du Bois.

Lee and Wetzstein (1990) carried out a study on *in vitro* propagation of muscadine grape by axillary shoot proliferation and reported that higher concentration of BAP (more than 10 µM) in the MS medium is known to inhibit shoot elongation in muscadine grape.

Thidiazuron (TDZ) at concentration of 2-4.5 µM was found to be effective for *in vitro* shoot proliferation in muscadine grapes. However, addition of BAP or kinetin at 1.0-5.0 µM in combination with TDZ increased the number of shoots production. (Sundarsono and Goldy, 1991).

In a study on micropropagation of thirteen *Malus* cultivars and rootstocks and the effect of antibiotic on proliferation, results indicated that highest shoot proliferation was obtained for rootstock produced 11.6 + 2.5 shoots (1.5 + 0.8 cm in length) per tube per month (Yepes and Aldwinckle, 1994).

In an experiment on *in vitro* propagation of a semi dwarfing cherry rootstock, it was observed that multiplication rate of about 6 was achieved over a 4 week period using MS medium augmented with 4.44 µM BAP and 0.49 µM IBA (Muna *et al*., 1999).

In a study on micropropagation of thirteen *Malus* cultivars and rootstocks and the effect of antibiotic on proliferation, results indicated that highest shoot proliferation was Half MS medium supplemented with BAP (2 mg/l) and IBA (0.02 mg/l) induced highest shoot regeneration frequency in Kyobo grape (Kwon *et al*., 2000). Maximum numbers of shoots in grape cv. Calmeria was obtained on B_5

medium containing 1.0 mg/l BAP, 0.05 mg/l IAA and 1.0 mg/l PP 333 (Jiang *et al*., 2000).

Vijayan *et al*. (2000) developed a protocol for plant regeneration from leaf explants for tropical mulberry varieties to study the effect of sugars, 6-benzyladenine and genotype on shoot regeneration. Highest percentage of shoot regeneration (80 ± 6) was obtained with genotype S799 on medium containing glucose and 8.9 µM 6-benzyladenine.

MS culture medium supplemented with NAA and BAP, Perola pineapple plants showed highest multiplication rate (13.5 shoots) with the smallest shoots inoculated as compared to MS medium free of growth regulators (Vescoa *et al*., 2014).

Bommineni *et al*. (2001) carried out an study on new method for rapid *in vitro* propagation of apple and pear with an aim to develop a rapid shoot multiplication procedure for clonal propagation and reported that cultured stem slices of apple cv. Gale gala and pear cv. Bartlett showed induction of shoots (37% and 97%) in MS medium supplemented with thidiazuron compared to medium augmented with BAP and kinetin.

Rahman *et al*. (2002) carried out a study on *in vitro* rapid clonal propagation of pineapple, *Ananas Comosus* (L.) Merr. Results from the study indicated that maximum number of multiple axillary shoot was proliferated on MS basal medium containing 1.0 mg/l BA and 0.1 mg/l NAA.

Ahmad *et al*. (2003) carried out a study on the effect of culture medium and growth regulators on micropropagation of peach rootstock GF677 and reported that BAP 0.6 mg/l produced higher number of shoots having > 2 cm length. Higher levels of BAP (0.9 mg/l) induced callus formation and shoot apical necrosis.

Kaushal *et al.* (2005) carried out an experiment for rapid clonal multiplication of apple rootstock MM111 using axillary buds with an aim to investigate the highest shoot proliferation rate using different concentration of BA (0.5-1.0 mg/l), GA_3 (0.5 mg/l) and with or without IBA (0.05-0.1 mg/l). Results indicated that MS medium supplemented with BAP (1.0 mg/l) and GA_3 (0.5 mg/l) gave highest multiplication rate (1: 5) and produced longer shoots (1.0-4.0 cm).

Dalal *et al*. (2006) reported maximum number of shoots (6.8 + 0.80) and maximum shoot length (17.5 + 4.6 mm) with highest sprouting percentage (85.0%) in the MS medium augmented with 2.22 µM BAP. They also reported that on repeated third subculture, maximum number of shoots (501.0 + 35.6) and shoot length (29.9 + 4.5 mm) was noticed in medium augmented with BAP at 2.22 µM + IBA 0.49 µM + Kn 2.32 µM.

Maximum shoot proliferation was obtained in MS medium supplemented with 1.0 mg/l BAP + 2.0 mg/l kinetin and 1.0 mg/l BAP + 1.0 mg/l kinetin in grape cultivars Perlette and Flame seedless, respectively (Kumar *et al*., 2006).

Venkatachalam *et al*. (2007) carried out a study with an aim to investigate the effects of high levels of cytokinins on micropropagation in banana. Results from the study revealed that the number of shoots increased with an increasing concentration of BAP up to 22.2 µM, and with higher levels of BAP (33-44.4 µM) a reduction in number of shoots regeneration occurred.

Kumar *et al*. (2008) obtained highest shoot proliferation rates in grape on MS medium augmented with 1.0 mg/l BAP and 1.0 mg/l kinetin. They obtained highest number of shoots (4.55) with a maximum shoot length of 2.20 cm with 1.0 mg/l BAP and 1.0 mg/l kinetin.

Ciccotti *et al*. (2009) studied different media *viz*., MS, QL, WPM and DKW used for multiplication of *Malus seiboldii* and reported that MS medium with an addition of iron showed highest proliferation rates, shoot number (4.8 shoots per explant) and shoot height (2.4 cm).

Micropropagation of Douce de Djerba apple through *in vitro* culture of axillary buds was carried in MS medium supplemented with BAP at 1.0 mg/l plus IBA at 0.1 mg/l and experiment revealed that MS medium supplemented with 1.0 and 2.0 mg/l produced multiple shoots (Boudabous *et al*., 2010).

Zou (2010) developed an effective *in vitro* culture system for mature stem segments of Chinese plum cultivar Gulf-ruby. He reported that the successful shoot multiplication was achieved on WPM medium with 0.05-1.0 mg/l IBA, 0.2 mg/l BAP, 0.3 mg/l KT and 1.0 g/l casein hydrolysate. They also reported that elongation of the shoot *in vitro* was facilitated on WPM medium 0.05 mg/l IBA, 0.3 mg/l KT/BAP and 1.0 g/l casein hydrolysate.

Haripyaree *et al*. (2011) reported that shoot tips of *citrus megaloxycarpa* produced multiple shoot buds when cultured on agarized MS medium containing 0.25 to 2.0 mg/l BAP alone and in combination with 0.50 mg/l NAA or with 0.50 mg/l kinetin.

Ruzic *et al*. (2011) carried out a study on *in vitro* growth responses of the Pyrodwarf pear rootstock to cytokinins using different concentrations (1, 2, 5, 10, and 15 µM of BAP and thidiazon) combined with IBA at concentrations of 0, 0.5, 205, and 4.9 µM in MS medium. Results of the study revealed that the highest multiplication index (2.89) was obtained on the medium supplemented with 5 µM BAP and 0.5 µM IBA.

Amiri and Elahinia (2011) carried out a study on optimization of medium composition for apple rootstocks with an objective to determine the impact of different growth regulators on shoot production *in vitro*. Results from the study indicated that best shoot production in terms of shoot number and shoot quality was obtained using 4.4 µM BAP and 2.27 µM thidiazuron (TZD) during the shoot multiplication phase. They also reported that highest shoot multiplication rate (5.7) and highest amount of total fresh weight (2.25 g/jar) was recorded with 4.4 µM BAP + 2.27 µM TDZ for M27 as compared to MM106 with lowest multiplication rate (0.7) per month.

Kour and Singh (2012) conducted an experiment for rapid *in vitro* propagation of rough lemon from nodal segments to standardize the protocol for mass multiplication under laboratory conditions. They reported that MS medium supplemented with BAP (1.5 mg/l) + malt extract (500 mg/l) resulted in maximum number of shoots (5.34), length of the shoot per culture (3.57 cm) in minimum time during shoot proliferation.

An experiment on efficient axillary shoot proliferation and *in vitro* rooting of apple cv. Topaz using QL medium macro elements and MS medium micro elements noticed that medium supplemented with 1.0 mg/l BAP + 1.5 mg/l kinetin produced similar types of shoots per explant (2.5 and 2.4, respectively). They also reported that medium augmented with 1.0 mg/l thidiazuron (TDZ) produced significantly higher number of shoots (3.6) but they were fasciated (Keresa *et al*., 2012).

In a study carried out on low-cost tissue culture procedures for micropropagation of apple rootstocks M9 using axillary buds, MS medium

supplemented with 3.0 mg/l BAP, 2.0 mg/l kinetin, 3.0 per cent sucrose and 0.8 per cent agar-agar (w/v) produced 85.70 per cent of shoot apices forming multiple shoots (Bhatt *et al*., 2012).

In an experiment carried out on *in vitro* propagation of AZ × M9 Irani apple rootstock, indicated that 0.4 mg/l BAP + 0.1 mg/l NAA supplementation in MS medium was found to be the best treatment that resulted in highest shoot proliferation rate (6.73) and highest shoot length (3.0 cm) was recorded on MS medium supplemented with 0.8 mg/l BAP (Tabalvandani *et al*., 2014).

Ghanbari (2014) carried out a study on impact of plant growth regulators and culture medium on *in vitro* propagation of three apple (*Malus* × *domestica* Borkh.) rootstocks *viz.,* Azayesh-Esfahan, Morabbaee-Mashhad and M9. Results from the study revealed that maximum shoot proliferation was recorded on MS medium supplemented with 1.5 mg/l BAP, whereas the minimum shoot proliferation was recorded in MS medium supplemented with 0.5 mg/l BAP. They also reported that rootstocks Azayesh-Esfahan, Morabbaee-Mashhad produced maximum and minimum shoot proliferation values (4.60 and 3.66 shoots per explant), respectively.

In the micropropagation of banana cv. Matti, results revealed that maximum number of shoot initials (8.66 + 0.58/explant) were obtained in the basal MS medium supplemented with 2.0 mg/l of BAP and kinetin, whereas zeatin and adenine sulphate showed the maximum shoot initials per explant in 2.5 mg/l BAP (Lohidas and Sujin (2015). Maximum proliferated cultures (95.30%) and shoot length (42.97 mm) in apple explants were recorded in woody plant medium (WPM) medium fortified with 3.0 mg/l BAP (Hu, 2015).

Kassaye and Bekele (2015) carried out a study on *in vitro* optimization of the protocol for micropropagation of plum cv. Methley from nodal explants and results revealed that highest shoot induction response (92%) was observed on MS medium supplemented with 0.5 mg/l BAP and 0.1 mg/l IBA with an average number of 2.08 ± 0.14 shoots per explant and 2.3 ± 0.26 cm average shoot length.

In an experiment of effect of media and plant growth regulators on micro propagation of Myrobalan 29 C rootstock, highest shoot number and length of shoots were 5.58 and 2.50 cm, respectively were obtained in MS medium supplemented with 2.0 mg/l BAP (Shabani *et al*., 2015).

Panchal and Patel (2016) carried out an experiment with an objective to develop commercially feasible and economically viable protocol for micropropagation technique for custard apple. Results revealed that in axillary bud explants, maximum frequency of multiple shoots (76.17%) was registered with MS medium + 2.0 mg/l BAP and 0.5 mg/l kinetin. Similarly, shoot tip explants performed better for frequency of multiple shoots (59.67%) in MS medium supplemented 2.0 mg/l BAP along with 0.5 mg/l kinetin.

In a study conducted on surface sterilization and *in vitro* propagation of *Prunus domestica* L. cv. Stanley using axillary buds as explants, the highest shoot induction was observed on MS medium fortified with 0.5 mg/l BAP in combination with 0.1 mg/l IBA with an average number of 3.08 ± 0.58 shoots per explants and 3.33 ± 0.29 cm average shoot length (Wolella, 2017).

Meneguzzi *et al*. (2017) carried out a study on micropropagation of apple rootstock G814 with an aim to develop a suitable protocol for micropropagation. Results from the study showed that BAP at 1.0 mg/l gave best results at multiplication phase.

Lizarraga *et al*. (2017) reported that highest shoot initiation rates were achieved in apple cultivar Principe Grande (55%) and Barburina pear cultivar (50%) on MS medium supplemented with cytokinin (0.5 mg/l and 1.0 mg/l) in combination with TDZ. Further, they reported that for all cultivars of apple and pear, the highest multiplication rates were obtained with medium supplemented with TDZ (1.0 mg/l) and also in medium containing BAP (1.0 mg/l).

Sharma *et al*. (2017) carried out a study to establish efficient and reliable *in vitro* propagation protocol for Gisela 5 - clonal cherry rootstock. Results from the study indicated that shoot multiplication and shoot length was increased with the increase in number of passages of sub culturing to a maximal of 1:10 and 6.5 cm, respectively after fourth passage.

In a study, development of tissue culture system for pear plants, Sedlak and Paprstein (2017) reported that the highest shoot proliferation rate was obtained for Bitterbrine Lucas pear plants that produce 4.9 shoots *in vitro* on MS medium augmented with 4.0 mg/l BAP.

Hassan and Zayed (2018) reported that supplementation of culture medium with BAP (0.5 mg/l) and IBA (0.25 mg/l) induced highest number of shoots after 30 days of culture in *Jatropha Curcas*. However, only IBA @ 1.0 mg/l induced the shoot elongation.

Rajoriya *et al*. (2018) carried out the study to determine the effect of plant growth regulators on the micro propagation as well as on the biochemical estimation of red banana. They revealed that, 3.0 mg/l BAP + 0.2 mg/l NAA showed highest shoot initiation and multiplication. They also reported that BAP @ 3.0 mg/l along with 0.2 mg/l NAA resulted in maximum number of shoots (5) as well as length of the shoot (9.0 cm).

Bhaya and Salim (2019) carried out a study to determine the impact of plant growth regulators and light quality to propagate banana (*Musa* spp.) cv. Dwarf Cavendish. Results revealed that 0.1 mg/l TDZ + 1.5 mg/l NAA gave highest shoot length (4.87 cm), whereas combination of 4.0 mg/l BAP + 1.5 mg/l NAA gave minimum length of shoots (1.37 cm).The shoot proliferation in Red Fuji apple shoots using nodal stem segment after 15 and 45 days of culture on MS medium fortified with 4.44 µM BAP and 0.05 NAA resulted in maximum shoot proliferation percentage as compared to other treatment combinations (Renu *et al*., 2018).

2.5 *In-vitro* rooting

Good quality of root induction *in vitro* is a known phenomenon due to plant growth regulators like auxins. *In vitro* rooting is a critical phase of micropropagation as it depends on the genotype and physiological condition at the time of root induction (Martins and Pedrotti, 2001).The plant growth regulators (IAA, IBA and NAA) have been popularly considered as rooting hormones in plant tissue culture technique. It is also widely accepted that auxin is necessary for root induction, but its continued presence in the medium may inhibit the development of adventitious roots (Dobranszki and Silva, 2010).

In an experiment on *in vitro* propagation of Malling 7 apple rootstock, Werner and Boe (1980) obtained better rooting in M7 apple rootstock in one third strength of MS medium supplemented with 2.0 mg/l IBA and 0.27 per cent agar within 28 days of culture.

Nemeth (1981) tried various synthetic auxins and reported that 2-chloro-3-(2,3 dichlorophenyl) propionitrile at 5 µM was found to be more effective than IBA for stimulating the rooting in M26, M27, MM104 and Stark spur.

Pua *et al*. (1983) obtained 100 per cent rooting in shoot cuttings of Ottawa 3 apple rootstock after 2 weeks in half strength MS medium supplemented with 6.25 mg/l IBA.

Hutchinson (1984) tried various auxins for root induction in apple cv. Northern Spy. It was found that roots can be readily induced with 1 µM IBA, but salts concentration and physical support on which shoot tips are grown influence both root initiation and growth.

Eng-Chong (1985) carried out a study to determine the impact of different carbon sources *viz.*, sorbitol, sucrose, glucose and fructose on *in vitro* rooting of Ottawa 3 apple rootstock and reported that 100 per cent rooting was obtained in MS medium containing 30 g/l sucrose.

Castelli *et al*. (1986) carried out an experiment to study the comparative response of standard and spur type apple cultivars to growth regulators under *in vitro* culture conditions and obtained highest rooting percentage by dipping apple propagules for 30 minutes in 400 mg IBA.

Klerk *et al*. (1993) reported that 3 µM IBA gave best rooting percentage in micro cuttings of *Malus* (Jork 9), whereas presence of BAP and 2,4-D in nutrient medium inhibit rooting. Arello *et al*. (1991) obtained 100 per cent rooting in MM111 shoots using MS medium supplemented with 0.1 mg/l NAA.

In a study on micropropagation of thirteen *Malus* cultivars and rootstocks and the effect of antibiotics on their proliferation, Yepes and Aldwinckle (1994) reported that lower concentration of IBA (1.0 mg/l) were found to be best for inducing rooting in liquid rather than in solid MS medium.

Modgil *et al*. (1994) reported 1.5 per cent sucrose in combination with 0.3 mg/l IBA under dark conditions for 7 days and activated charcoal containing MS medium resulted in 70-80 per cent rooting in apple cv. Golden Delicious.

Correa *et al*. (1994) carried out a study to determine the impact of IBA and mineral salts on *in vitro* rooting of apple rootstock M793 and reported that during

explants (1-2 cm) culturing on MS medium adjusted to mineral salt concentration of 0, 25, 50, 75 or 100 per cent of the original level, best rooting (85%) was obtained with 1.0 mg/l IBA + 100 per cent mineral salts.

Aklan *et al*. (1997) reported 90 percent rooting in apple rootstock MM106 in MS medium supplemented with 5μM IBA and 162 mg/l phoroglucinol (PG) under dark conditions for 4 days. Alvarez *et al*. (1989) study the relationship between IBA levels in apple rootstocks cultured *in vitro* and reported that IBA (4.0 μM) induced maximum rooting percentage (80% and 100%) in M9 and M26, respectively.

Das *et al*. (2000) reported that regenerated shoots of sweet orange rooted best in MS medium supplemented with either NAA (0.75 mg/l) or NAA (0.5 mg/l) and IBA (2.0 mg/l).

Naik *et al*. (2000) observed maximum rooting percentage (92%) and number of roots (10.3 roots/shoot) in half strength MS medium supplemented with 0.54 mM NAA, whereas nutrient medium augmented with 0.054-5.4 mM NAA took minimum number of days (10 ± 15) for root initiation in pomegranate.

A study was carried out to determine the influence of aromatic cytokinins (6-benzylaminopurine (BAP), 6-benzyl amino purine riboside, meta-topolin and kinetin on shoot multiplication and their effect on rooting of apple cv. Husvetirozmaring. Results of the study revealed that 0.5 mg/l BAP increased the number of roots while meta-topol inincreased the length of the roots (Dobranszki *et al*. (2000).

In vitro raised shoots of Mosambi cultivar Jaffa showed good response for *in vitro* rooting on half strength MS medium supplemented with 0.5 mg/l NAA combined with 0.5 mg/l IBA. Further, half strength MS medium supplemented with 0.5 mg/l NAA combined with 0.5 mg/l IBA resulted in longest root (5.33 cm) in Jaffa mosambi plantlets (Krishan *et al*., 2001).

Azad and Amin (2001) carried out a study on rapid clonal propagation of banana (*Musa* sp.) using *in vitro* culture of floral bud apex and obtained satisfactory rooting percentage and number of roots in half strength MS medium supplemented with 2.0 mg/l IBA.

In a study, Jun *et al*. (2001) during multiplication of M9 apple rootstocks *in vitro* reported best rooting percentage in half strength MS medium supplemented with 0.5 mg/l IBA, 20 g/l sucrose and 8.0 g/l agar.

Kaya and Gubbuk (2001) conducted a study on *in vitro* propagation and rooting in some citrus rootstocks through tissue culture in Troyer citrange and Carrizo on MS medium supplemented with 1.0 mg/l BAP + 1.0 mg/l NAA + 1.0 mg/l GA_3 . Results from the study revealed that MS medium supplemented with NAA and GA_3 obtained maximum rooting percentage as compared to BAP alone.

Al-Khayri and Al-Bahrany (2001) obtained highest percentage of rooting (56%) in MS medium supplemented with 1.0 mg/l IAA in *Citrus aurantifolia*. They also reported that presence of 2.0 mg/l IAA in MS medium produced longest roots than other hormone concentrations.

Magyar *et al*. (2002) carried out a study to investigate the rooting response of apple rootstocks *viz.,* JTEH, M26 and MM106 at different levels of IBA (1.0, 2.0, 3.0 mg/l) in root induction medium (RIM) and presence of activated charcoal (2.5 g/l) in the root elongation medium (REM). Rootstock JTEH showed very high rooting capacity at each IBA level (90%), whereas MM106 showed very poor rooting percentage (33-46%). They also reported that in M26, the higher rooting percentage (94%) was obtained with lower IBA levels.

Rahman *et al*. (2002) reported that the number of roots produced per plantlet increased with the increasing concentration up to 2.0 mg/l IBA and declined thereafter up to 5.0 mg/l. Among the IBA treatments, 2.0 mg/l IBA was found to be the most effective in rooting of banana plantlets. Further, highest number of roots/plantlet was obtained in 2.0 mg/l IBA. However, rate of increase in root length was rapid in the media with 2.0 or 3.0 mg/l IBA but was slower in the media with 5.0 mg/l and control.

Al-Bahrany (2002) carried out a study on effect of phytohormones on *in vitro* shoot multiplication and rooting of lime *Citrus aurantifolia* and obtained highest rooting percentage in MS medium supplemented with 1 mg/l NAA or 0.5 mg/l NAA + 2 mg/l IBA, whereas maximum number of roots were produced in MS medium augmented with 2 mg/l NAA+ 2 mg/l IBA.

Rana and Singh (2002) carried out a study on *in vitro* clonal propagation of kagzi lime (*Citrus aurantifolia* Swingle) through shoot tips and reported that MS medium supplemented with 2.0 mg/l IBA gave highest rooting percentage in minimum number of days.

Ahmad *et al*. (2003) carried out a study on the effect of culture media and growth regulators on micropropagation of peach rootstock GF 677 and reported that best root system was developed on half strength MS media supplemented with IBA (3.0 mg/l). However, higher levels of IBA (4.0 mg/l) induced callus and inhibit normal root development.

Balla *et al*. (2003) conducted an experiment to investigate the effects of sucrose concentration in the rooting medium for JTEF apple rootstock and revealed that MS medium supplemented with 60.0 g/l sucrose and 5 mg/l IBA gave maximum rooting (71%). Begum *et al*. (2003) obtained highest rooting percentage (100%) in Pummelo (*Citrus grandis*) in half strength MS medium containing 0.1 mg/l IBA.

Karwa (2003) revealed that MS medium supplemented with 4.92 µM IBA induced rooting up to 50.25 per cent, whereas highest rooting percentage (78.44%) and average number of roots per shoot (5.8 ± 0.86) were obtained in MS medium supplemented with 4.92 µM IBA + 1.11 µM BAP in Nagpur mandarin.

Molla *et al*. (2004) carried out a study on *in vitro* rooting and *ex vitro* plantlet establishment of BARI Banana 1 (*Musa* sp.) as influenced by different concentration of IBA. Results revealed that an average of 8.28 roots per plantlet and length of the longest root (2.60 cm-5.67 cm) was obtained in MS medium supplemented with 0.5 mg/l IBA.

Dalal *et al*. (2006) recorded maximum root initiation (75.90%) and least callusing in clonal apple rootstock M9 with full strength MS medium augmented with IBA (14.70 µM).

The highest rooting percentage (94.00%) was obtained with lowest IBA concentration. The two step rooting was found better in apple rootstocks MM111, by transferring micro cuttings to auxins free solid medium after root initiation on half strength MS medium containing 0.5 mg/l IBA (Kaushal *et al*. 2005).

The root elongation phase has been found to be very subtle to auxin concentration, high concentration of auxin impeded the root elongation and a medium concentration of IBA supports the induction of root in banana explants (Ganapathy *et al*., 2005).

Sumaryono *et al*. (2004) reported early rooting of banana shootlets in MS medium supplemented with 2.5 mg/l of IBA, NAA and 0.5 mg/l AC.

Shoots of apple rootstock raised *in vitro* were transferred to various rooting media to study the impact of different factors on root initiation and development. Various concentration of IBA initiate rooting but maximum rooting percentage was observed with 2.0 mg/l and 2.5 mg/l of IBA in M7 and with 1.0 mg/l of IBA in MM106. Further, presence of activated charcoal in rooting medium improved rooting quality but reduces rooting percentage in both apple rootstocks (Sharma *et al*., 2007).

In a study, effect of growth hormones in the micropropagation of banana cv. Matti, Dihiz *et al*. (2007) reported that more number of roots are produced in *in vitro* raised banana shoots with MS medium supplemented with 0.5 mg/l IBA, 0.5 mg/l NAA and 2 g/l activated charcoal.

During study of micropropagation of apple proliferation-resistant apomictic *Malus seiboldii* genotypes, Ciccotti *et al*. (2009) reported that higher rooting percentage was observed when induction was carried out under dark conditions with IBA at 25 µM either in liquid or agarised MS medium.

In an experiment on *in vitro* multiplication of Merton I. 793- An apple rootstock suitable for replantation, it was observed that better rooting percentage (77.00%) was observed in MS medium (1/2 strength) supplemented with NAA at 0.1 mg/l (Soni *et al*. 2010).

Saini *et al*. (2010) reported that highest rooting percentage (77.00%) was observed in MS medium fortified with 1.0 mg/l NAA combined with 1.0 mg/l IBA in *Citrus jambhiri.*

Modgil *et al*. (2010) carried out a study on *in vitro* rooting and hardening in apple rootstock EMLA111 with an aim to develop efficient and reliable procedure for rooting hardening. Results revealed that MS medium supplemented with IBA and NAA had a significant effect on rooting of *in vitro* raised shoots and maximum rooting (75%) was observed with 0.3 mg/l IBA.

In a study on micropropagation of chinese plum (*Prunus saliciana* Lindl.) using mature stem segments, Zou (2010) reported that half strength MS medium supplemented with 0.2-0.5 mg/l IBA, 15 g/l sucrose and 20-40 mg/l phoroglucinol (PG) proved best for higher rooting percentage (10.42%) without phoroglucinol and 79.76 per cent with added phoroglucinol in the half strength MS medium.

Modgil *et al*. (2010) during development of an efficient and reliable procedure for *in vitro* rooting and hardening of *in vitro* raised shoots of EMLA111 indicated that nearly 75 per cent rooting was obtained in ½ MS medium with 0.1–0.5 mg/l IBA, 2 per cent sucrose and 0.4 per cent agar in one step method.

Boudabous *et al*. (2010) carried out a study on micropropagation of apple cultivar Douce de Djerba) through *in vitro* culture of axillary buds and reported that high frequency of rooting (66.70%) with development of healthy roots was observed from shoots cultured on half strength MS medium enriched with IBA (3.0 mg/l) and 2.0 mg/l of activated charcoal.

Different *in vitro* regenerated shoots of pear rootstocks were subjected to rooting in half strength MS medium encompassing 1.0 mg/l IBA and 0.1 GA_3. Rootstock Ferely Jaspi showed better rooting percentage (100%), highest number of shoots (8.1) and longest root length (3.08 cm) as compared to the other rootstocks (Vujovic, 2012).

An experiment was carried out to examine the effects of different combinations of plant growth regulators on *in vitro* micropropagation of strawberry. Results revealed that maximum number of roots per explant was obtained in MS medium supplemented with0.1 mg/l BAP along with IBA 0.2 mg/l auxin (Moradi, 2011).

In a study, Ruzic *et al*. (2011) reported that half strength MS medium supplemented with 5 µM IBA and 0.3 µM GA_3, produced highest rooting percentage (96%), root numbers (4.88) and length of the longest root (1.83).

Amiri and Elahinia (2011) carried out a study on optimization of medium composition for apple rootstocks with an aim to determine the impact of different growth regulators on root production. Results indicated that half-strength MS medium containing 5.4 µM IBA and 1.2 µM 2,4-D provided significant root production. They also reported that highest rooting percentage (64%) was produced in MM106 genotype and lowest (11%) in M9 after 3 months.

The separated shoots of *Citrus magaloxpcarpa* were rooted and elongated on MS medium containing 1.0 to 2.0 mg/l IAA, IBA and NAA. Whereas, NAA at 0.50 mg/l in combination with BAP at 0.25 mg/l was found more effective in induction of the roots (Haripyaree *et al*., 2011).

Kour and Singh (2012) carried out a study for rapid *in vitro* of rough lemon from nodal segments to standardize the protocol for mass multiplication under laboratory conditions using half strength MS medium supplemented with IBA (1.0 mg/l) and NAA (1.0 mg/l) alone or in combination and sucrose (1.5, 3.0 and 4.5%). Results from the study revealed that *in vitro* shoots were best rooted in half strength MS medium supplemented with IBA (1.0 mg/l) + NAA (1.0 mg/l). They also reported that IBA (1.0 mg/l) and NAA (1.0 mg/l) obtained highest rooting percent (83.33%), number of root per shoot (2.47) and length of longest root (3.57 cm).

Miilion *et al*. (2013) carried out an experiment with an aim to study the impact of different concentrations of growth regulators (IAA and IBA) on rooting in banana cultivar Grand Naine. Results indicated that minimum number of days (8.9) taken for root initiation, number of roots (6.2) and length of the longest root (5.7 cm) was recorded in MS medium supplemented with 0.5 mg/l IAA as compared to different concentrations of IBA.

The half strength MS medium supplemented with 2.4 g/l thiamin + 0.15 g/l Fe-EDDHA was found to be the best component that resulted in the highest root number (3.5) and root length (3.25 cm) in Irani apple hybrid rootstock (AZ × M9) (Tabalvandani *et al*., 2014).

Aygun and Dumanoglu (2015) carried out an experiment for *in vitro* root formation of *Pyrus elaegrifolia* Pallas. Results indicated highest rooting rate (54.2 + 10.4%), root length (10.5 + 2.4 mm) and root number (2.5 + 0.6) under 10 days dark treatment in the medium containing half strength of macronutrients supplemented with 5 µM IBA.

Shabani *et al*. (2015) in an experiment on effect of media and plant growth regulators on micro propagation of Myrobalan 29C rootstock observed that, Driver and Kuniyaki Walnut (DKW) medium supplemented with 1.0 mg/l NAA resulted in highest rooting (100%). However, medium supplemented with 2 mg/l NAA resulted in maximum root length (14.5 cm).

Lohidas and Sujin (2015) during study on effect of growth hormones in the micropropagation of banana cv. Matti reported that minimum number of days (08.33 ± 0.58) were taken for root initiation, number of roots (9.33 ± 0.58) and root length (5.23 ± 0.12 cm) was observed in MS medium supplemented with IBA @ 2.5 mg/l.

Kassaye and Bekele (2015) reported that the highest percentage of rooting was observed with MS medium supplemented with 1.0 mg/l IBA in *Prunus saliciana* L. cv. Methley. IBA @ 1.0 mg/l was also found effective for average number roots and root length were 4.34 ± 1.15 and 3.00 ± 0.08 cm, respectively. Moreover, increase in the concentrations of IBA decreased the rooting ability of *Prunus saliciana* L. cv. Methley.

The maximum rooting percentage (13.34%) was observed in MS medium supplemented with IBA (0.1 mg/l) followed by NAA (1.0 mg/l) with rooting percentage of 29.61 per cent in *in vitro* raised shoots of apple. Moreover, maximum roots per explant (3.40) were obtained on MS medium supplemented with 1.0 mg/l NAA (Hu *et al*., 2015).

Lazo-Javalers *et al*. (2016) carried out a study on surface disinfection procedure and *in vitro* regeneration of grape vine cultivar Flame seedless and reported higher percentage of rooting in Flame seedless grape vine plants in half strength MS medium containing 2.0 mg/l IBA in combination with 200 mg/l activated charcoal.

Meneguzzi *et al*. (2017) carried out the study to standardize the protocol for new apple rootstock series G. 814 using QL media supplemented with different concentration of IBA (0.5, 1, 1.5, 2 and 2.5 mg/l) for successful rooting and the study revealed that IBA @ 2.5 mg/l resulted in highest rooting percentage (80%) as well as length of the root (5 cm).

A study was carried out on *in vitro* propagation of eight apple and two pear cultivars. Results from the study revealed that MS medium with 0.1 mg/l IBA without cytokinins showed best rooting percentage (91%) in case of Camoesa and Repinaldo cultivars (Lizarraga, 2017).

Sharma *et al*. (2017) carried out a study to establish efficient and reliable *in vitro* propagation protocol for Gisela 5 (*Prunus cerasus* × *Prunus canescens*) - clonal cherry rootstock and obtained maximum *in vitro* rooting (100%) on full strength MS medium fortified with 0.5 mg/l IBA. They also reported that highest number of roots and root length was observed on full strength solid MS medium supplemented with 0.5 mg/l IBA followed by 1.0 mg/l NAA and 0.5 mg/l IAA.

In a study conducted on surface sterilization and *in vitro* propagation of *Prunus domestica* L. cv. Stanley using axillary buds as explants with an aim to

investigate the factors needed for establishing an effective protocol for propagation. Results from the study reported that the highest rooting was observed on half strength MS medium augmented with 1.0 mg/l IBA with an average number of 4.25 ± 1.2 roots per shoot and 3.6 ± 0.1 cm average root length (Wolella, 2017).

Renu *et al*. (2018) reported that out of the different concentrations of NAA tested for Golden Delicious apple plants, 0.53 µM NAA supported highest rooting (average of 7.66 number and length of 22 cm) followed by 0.05 µM NAA where, an average of 6.33 root number and 20 cm length were obtained.

Rajoriya *et al*. (2018) carried out the study to determine the effect of plant growth regulators on the micro propagation as well as on the biochemical estimation of red banana. Results from the experimental study revealed that IBA (2.0 mg/l) along with NAA (0.5 mg/l) resulted in maximum rooting (100%). Maximum number of roots (4) as well as maximum root length (4 cm) was observed with IBA (2 mg/l) + NAA (0.5 mg/l).

Podwyszynska and Cieslinska (2018) noted that the use of putrescine, a polyamine, when used in combination with NAA and IBA, was able to enhance the rooting of neo-tetraploids of four apple cultivars *viz.*, Free Redstar, Gala Mast, Pinova and Red chief.

2.6 Acclimatization/ Hardening

Acclimatization is the adaptation of the *in vitro* rooted plantlets to the different environmental stress after their transplant. It is very important for *in vitro* raised plantlets because *in vitro* plant material is not readily adopted *in vivo* conditions (Brainerd and Fuchigami, 1981).

Hutchinson (1984) achieved maximum survival percentage (80%) in Northern Spy planlets using coarse sand due to better aeration rather than peat moss: coarse sand (1:3) due to poor drainage, with intermittent misting or anti transpirant spraying.

Le and Collet (1991) used perlite, peat, sand and optima for acclimatization of apple cv. Golden Delicious and M26 and MAC9 rootstocks. For Golden Delicious and MAC9, survival rate and plant growth was higher in optima + perlite and least in peat + perlite. Whereas, for M26, peat + perlite was found better.

In a study, Babylatha (1993) reported 90 percent survival rate in banana plantlets transplanted within two or three weeks after root initiation in culture room at 26 $\pm$ 2°C. They also reported that potting mixture containing sand: soil: vermiculite (1:1:1) gave maximum survival percentage (95%) under lath house conditions.

In vitro regenerated Chandler strawberry plants when covered with polythene sheets and plastic trays with 98 in-built small pots, highest survival percentage (80%) was observed in polythene bags filled with soil: FYM (1:1) during the hardening period (Kour and Chopra, 2004).

Molla *et al*. (2004) reported 95-100 percent survival of banana plantlets when they were transferred to small plastic pots after 15-20 days of *in vitro* culture in half strength MS medium augmented with 0.4-0.6 mg/l IBA and 7 days hardening at room temperature.

Kour *et al*. (2005) successfully established strawberry plantlets by washing five weeks old *in vitro* revived plantlets and transferring to plastic pots containing a pre autoclaved mixture of sand: soil: FYM (1:1:1). The pots were shifted under 28 $\pm$ 2°C and 60 per cent relative humidity for 15-20 days in green house for acclimatization and then shifted to fields successfully.

An experiment was carried out on *in vitro* multiplication of apple rootstock through culturing of shoot apices and axillary buds. Results indicated that *in vitro* rooted plantlets were successfully transferred paper cups containing peat within 3-4 weeks and acclimatized plantlets showed normal growth with no morphological variations (Kaushal *et al*., 2005).

In a study on *in vitro* cloning of apple (*Malus* × *domestica* Borkh.) employing forced shoot tip cultures of M9 rootstock, Dalal *et al*. (2006) reported that transfer of *in vitro* plants to polyvinyl cups or polybags under controlled descending RH regime of 95 per cent to 70 $\pm$ 5 per cent over a period of 5 $\pm$ 1 week, resulted in 80 per cent *ex vitro* survival.

Ciccotti *et al*. (2009) carried out an experiment on micropropagation of apple resistant apomictic *Malus Seiboldii* genotypes and reported that acclimatization protocol adopted for the *ex vitro* plants in the greenhouse yielded survival rates between 90-100 per cent.

During an experiment on micropropagation of Chinese plum using mature segments, results revealed that regenerated shoots were successfully acclimatized to greenhouse conditions and grew vigorously with no apparent phenotypic aberration (Zou, 2010).

Boudabous *et al*. (2010) carried out a study on micropropagation of apple cultivar Douce de Djerba using *in vitro* axillary buds. Results indicated that regenerated shoots of apple showed a frequency of 60 per cent survival after transferred to the field.

Soni *et al*. (2010) carried out a study on *in vitro* multiplication of Merton793 apple rootstock and the results revealed that 30 plantlets of Merton793 were successfully hardened after 7 weeks, with around 80 per cent survival rate.

Bhatt *et al*. (2012) carried out an experiment under low cost tissue culture procedures for micropropagation of apple rootstocks which can be used for replantation in apple orchards. During the experimentation, it was observed that out of different potting mixture composition i.e. sand: soil: vermiculite (1:1:1), sand: soil: perlite (1:1:1), sand: soil: cocopeat (1:1:1), sand: soil: perlite (1:1:1) and cocopeat (1:1:1:1:1), the highest survival rate (60.5%) was recorded in sand: soil: perlite (1:1:1). They also reported maximum shoot biomass (2.45 $\pm$ 0.23 g) and number of leaves (24.4 $\pm$ 0.23) in sand: soil: perlite (1:1:1).

Acclimatization under mist system in greenhouse of rooted shoots was successful (above 90%) for most of the plum rootstocks, with exception of Gisela 5 where shoots exhibited significantly lower potential (61.8%) to acclimatization (Vujovic *et al*., 2012).

Kour and Singh (2012) while working on *in vitro* multiplication of rough lemon (*Citrus jambhiri* Lush.) reported that out of different potting mixture *viz.*, soil (100%), soil + sand (1:1), soil + sand + FYM (1:1:1) and soil + sand + vermiculite (1:1:1), the maximum survival percentage (93.33%) was achieved in soil + sand + vermiculite (1:1:1).

Lalrinsanga *et al*. (2013) during a study on *in vitro* shoot tip culture of banana cultivar Meitei Hei reported 98-100 per cent survival rate in all the *ex vitro* hardening media of sand (100%), soil: FYM (2:1) and soil: vermicompost (2:1). They also

reported that survival rate of 98-100 per cent was observed in all the treatments but 100 per cent survival rate was observed only in river sand potting media.

In a study, Aygun and Dumanoglu (2015) reported that *in vitro* rooted plants of *Pyrus elaeagrifolia* Pallas showed good response during acclimatization period when transferred to the pots containing an autoclaved mixture of peat (70%), perlite (12%), sand (12%) and orchard soil (6%) covered with plastic film. Shabani *et al*. (2015) reported that maximum survival per cent (80%) was observed with substrate perlite (100% V) in Myrobalan 29C plum rootstock.

Sharma *et al*. (2017) carried out a study on efficient method for *in vitro* propagation of Gisela 5 (*Prunus cerasus* × *Prunus canescens*) - clonal cherry rootstock and reported that when rooted plantlets transplanted in sterilized sand for hardening and kept in the glasshouse, 90 per cent survival rate was observed after 4 weeks of transfer.

2.7 Economics of *in vitro* production of plantlets

Economics consideration assumes prodigious importance in any commercial attempts of mass multiplication of plants. However, very few workers have worked out the economics analysis of *in vitro* produced plantlets.

Rajmohan (1985) carried out a study on *in vitro* shoot tip culture technique in jackfruit and reported that on an average 65.83 plantlets could be produced per year from the single explant. The unit cost of one jackfruit plantlet including one month hardening was estimated to be ₹ 9.09.

Rajeevan and Pandey (1986) reported that by *in vitro* shoot tip culture in papaya, on an average 19,200 plants could be produced in every three weeks cycle from 250 initial shoot tips. They also reported that *in vitro* rooting band greenhouse stages are the most expensive items and estimated the cost of one papaya plantlet ready for field planting to be ₹ 0.85. During *in vitro* propagation studies of banana and papaya, Babylatha (1993) estimated the unit cost of one banana plantlets to be ₹ 2.22 including four weeks of greenhouse stage.

Wali (1996) reported that unit cost up to polygreen house stage of one guava plantlet obtained from seedling explant source was estimated to be ₹ 1.95, whereas

the unit cost of one guava plantlet obtained from mature tree explant source was estimated to be ₹ 2.05.

Matsumoto *et al*. (1998) carried out a study on micropropagation of santra orange and refinement of production procedure by cost simulation. Results revealed that one shoot explant of *Citrus reticulata* produced 1,80,000 shoots/year, while the cost of one *in vitro* grown plantlet was estimated to be 165 Yen.

Study was carried out on economic analysis of tissue cultured and sucker propagated banana in Tamil Nadu suggested that tissue cultured banana is more profitable to the farmers than sucker propagated banana Alagumani (2005).

Kour *et al*. (2009) conducted an experiment on economics of *in vitro* and *ex vitro* grown plantlets of *Citrus jambhiri*. Experimental results revealed that the unit cost of one *in vitro* grown *Citrus jambhiri* plantlets obtained from nodal segment explant excised from 8-10 year old mature tree was estimated to ₹ 2.54, whereas the estimated production cost of *ex vitro* was double i.e. ₹ 4.48.

In an economic analysis of tissue cultured and sucker propagated banana in India, Bachchan (2016) reported that tissue cultured banana is more profitable to farmers than sucker propagated banana. Moreover, per hectare yield in tissue culture banana was 60.50 metric tonnes which was higher than the yield of sucker banana (45.50 metric tonnes). Results also revealed that the total cost of production of tissue culture banana was ₹ 210035 per hectare, which was higher than the cost of production of sucker propagated banana ₹ 163306.

Chapter-III

Materials and Methods

CHAPTER-III

MATERIALS AND METHODS

The present investigation entitled "Propagation studies on clonal rootstocks of apple (*Malus* × *domestica* Borkh.)" using two clonal apple rootstocks MM106 and MM111was carried out at Plant Tissue Culture Laboratory of School of Biotechnology and Tissue Culture Laboratory, Advanced Centre for Horticulture Research (ACHR), Udheyawalla of Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu, Chatha during the year 2018. The details of materials used and experimental procedures adopted during the course of present investigation is presented in this chapter as under

3.1 Climate and weather conditions of site

SKUAST-Jammu, Chatha is located at 32.73°N latitude 74.87°E, longitude and elevation of 300 meters (980 ft) from mean sea level. Jammu is surrounded by the Shivalik range in the north, east and southeast while the Trikuta range surrounds it in the north-west.

Udheywalla, Jammu is situated in the sub-tropical zone at latitude of 32.43°N and longitude 74.51°E longitude. The altitude of the place is 300 meters from mean sea level.

3.2 Experimental details

3.2.1 Plant material

A study was conducted to standardize the protocol for *in vitro* propagation and to find out the economics of *in vitro* propagation of apple clonal rootstocks MM106 and MM111. The explant material was collected from Regional Research Horticulture Sub-Station (RRHSS), Bhaderwah, SKUAST-Jammu. Two types of explants *viz.*, shoot tip and nodal segment were used for the experimental study. The explants were collected and cultured in different months during the year 2018.

3.2.2 Chemicals

Throughout the investigation, precautions were taken to use chemicals of high purity only. Chemicals such as growth regulators, vitamins, myo-inositol, sucrose etc. were obtained from reliable firms like Hi-media, SMD (Star Micronics Devices) and other standard firms.

3.2.3 Glassware

The glassware used was of boro-silicate quality and a product of Borosil India Pvt. Ltd.

3.2.4 Preparation of culture medium

In order to study the morphogenic response of clonal apple rootstocks (MM106 and MM111) explants, the most widely accepted MS medium (Murashige and Skoog, 1962) was used as a basal medium. The basal medium was prepared by mixing the required quantity of each of the four solutions as given in Table 3.1. The stock solutions number 1-4 of macro-nutrients, micro-nutrients, vitamins and Fe-EDTA were prepared by dissolving the required amount of salts in measured quantity of distilled water.

For preparation of culture medium, about 700 ml of distilled water was taken in a container (1000 ml). After that, required stocks were carefully measured by using correct sized pipette or graduated cylinder with a proper range balance. Then required amounts of myo-inositol (100 mg/l) and sucrose (30 g/l) were weighed and added to the beaker containing the stocks and water. The growth regulators were added according to the requirements. After all the additions were done, the ingredients were dissolved properly and distilled water was added to make the final volume. Then, pH was adjusted to 5.8 with the help of 0.1 N NaOH and 0.1 N HCl. For gelling the media, required amount (8 g/l) of agar was weighed and added. Agar was dissolved by boiling the media till it become homogenous. Then, medium about 35 ml and 16 ml was dispensed in glass jars (100 ml) and test tubes (25 × 150 mm), respectively and finally plugged by using non-absorbent cotton wrapped with muslin cloth. Medium was autoclaved at 15 lbs/inch^2 pressure for 15 minutes and then allowed for solidification at room temperature.

For preparing the media which contained polyvinyl pyrrolidone (PVP), citric acid, ascorbic acid or activated charcoal (AC), required amount of these materials was measured and added in the medium before adjusting its pH. After autoclaving, to prevent the suspension of undissolved polyvinyl pyridine and activated charcoal in medium, slight shaking of the culture jars and tubes was done.

Table 3.1: Preparation of stock (MS media)

Component	Chemical formula	Required concentration (mg/l) 1X	Volume 1000 ml (40X)
STOCK-1 (Macro nutrients) 40X			
Calcium chloride	$CaCl_2.2H_2O$	440	17.6 g
Potassium dihydrogen phosphate	KH_2PO_4	170.0	6.8 g
Potassium nitrate	KNO_3	1900.0	76 g
Magnesium sulphate	$MgSO_4.7H_2O$	370.0	14.8 g
Ammonium Nitrate	NH_4NO_3	1650.0	66 g
Note: Dissolve $CaCl_2$ separately and mix			
STOCK-2 (Micro nutrients) 400X			
Cobaltous chloride	$CoCl_2.2H_2O$	0.025	10 mg
Copper sulphate	$CuSO_4.5H_2O$	0.025	10 mg
Boric acid	H_3BO_3	6.200	2480 mg
Potassium iodide	KI	0.830	332 mg
Manganese sulphate	$MnSO_4.H_2O$	16.90	6760 mg
Sodium molybdate	$NaMoO_4.2H_2O$	0.250	100 mg
Zinc sulphate	$ZnSO_4.7H_2O$	8.600	3440 mg
STOCK-3 (Iron source) 200X			
Ferrous Sodium EDTA	FeNaEDTA	36.7	7.34 g
STOCK-4 (Organics) 400X			
Thiamine HCL	$C_{12}H_{17}CIN_4OS.HCL$	1	400 mg
Nicotinic acid	$C_6H_5NO_2$	0.5	200 mg
Pyridoxine HCL	$C_8H_{11}CINO_3.HCL$	0.5	200 mg
Myo-Inositol	$C_6H_{12}O_6$	100	40000 mg
Glycine	$C_2H_5NO_2$	2	800 mg

Note: All these stocks are prepared by dissolving the components one by one in double distilled water.

Table 3.2: Nutrients taken for MS media preparation

	1 litre	500 milliliter
Stocks-1	25 ml	12.50 ml
Stocks-2	2.50 ml	1.25 ml
Stock-3	5.0 ml	2.50 ml
Stock-4	2.50 ml	1.25 ml
Sucrose	30 g	15 g
Add growth regulator, if needed		
Make up the final volume		
Adjust the pH 5.8		
Agar-agar	8 g	4 g
Dissolve the agar by using oven and pour it into the inoculation glass jars/test tubes.		

3.2.5 Preparation of stock solution of growth regulators

The stock solution of growth regulator like cytokinin (like BAP) was prepared by dissolving 50 mg of BAP in few drops of 1 N NaOH. The final volume was made to 100 ml by adding distilled water. Likewise, the stock solution of GA_3, auxins (IBA and NAA) were prepared by dissolving 50 mg each of GA_3, IBA and NAA in a few drops of absolute alcohol and the final volume was made to 100 ml by adding distilled water.

Table 3.3: Plant growth regulators, their abbreviation and solvents used in the present investigation

Plant growth regulator	Abbreviation	Solvent
6-Benzylaminopurine	BAP	1 N NaOH
Gibberellic Acid	GA_3	Absolute ethyl alcohol
Indole Butyric Acid	IBA	Absolute ethyl alcohol
Naphthalene Acetic Acid	NAA	Absolute ethyl alcohol

3.2.6 Storage of stock solutions and culture media

The stock solution of growth regulators, amino acids and other chemicals such as glycine, nicotinic acid, MS major nutrients, MS minor nutrients, antibiotic etc. were prepared and stored in a refrigerator at 4 $\pm$ 1 ^{o}C in a glass bottles. Repeated freezing and thawing was avoided.

3.2.7 Culture vessels

Culture vessels *viz.*, glass jars (150 ml) and test tubes (25 mm × 150 mm) made of borosil glass were used as culture vessels. All the culture vessels and glassware used in the preparation of media and for other purposes were cleaned in chromic acid (potassium dichromate in sulphuric acid). The acid was removed by prolonged rinsing in tap water. They were then washed with 10 per cent teepol with a test tube brush and were thoroughly rinsed in tap water. After then glassware were finally rinsed with double glass distilled water and dried in oven at 70 ^{o}C.

3.2.8 Sterilization of media, cultural vessels and instruments

After the adjustment of pH, known volume of media was distributed in culture vessels (35 ml in culture flasks and 16 ml in test tubes) for autoclaving. The mouth of the culture vessels were plugged with non-absorbent cotton wrapped in gauze cloth. The mouth of the culture vessels were further covered with an ordinary paper to protect them from condensing water vapours during autoclaving of 15 lbs/in^2 for 15 minutes at approximately 121 ^{o}C. After sterilization, culture vessels were immediately transferred to an air-conditioned culture room.

All the instruments such as surgical scalpel handle (Size no. 4), disposable surgical blade (Size no. 20), forceps (20 cm in length) etc. were sterilized with methanol and sterilized (red hot) by flaming for repeated use. The instruments were also autoclaved from time to time to avoid any contamination.

3.2.9 Types and preparation of explant

The explants used were shoot tip and nodal segment from the juvenile branches of MM106 and MM111 clonal apple rootstocks planted at Regional Research Sub-Station (RHRSS), Bhaderwah (SKUAST-Jammu). Fifteen to twenty centimeter long bud wood shoots were taken from the current season's growth. These bud wood shoots were brought to the laboratory and washed in running tap water for 20 minutes. These bud wood shoots were further divided into shoot tips (0.5-0.75 cm) and nodal segments (0.5-0.75 cm) to be used as explants to see their influence on contamination, phenol exudation as well as bud brake.

3.2.10 Surface sterilization of explants

The different explants such as shoot tip and nodal segment were washed in running tap water for 20 minutes and then put in a conical flask containing 5-6 drops of Tween-20 for 15 minutes followed by bavistin treatment (0.2 %) for 20 minutes. After draining it off, explants were rinsed in sterile distilled water (SDW).

Then explants were surface sterilized with two different surface sterilants i.e. mercuric chloride ($HgCl_2$) and sodium hypochlorite (NaOCl) in separate treatments under aseptic conditions of laminar air flow cabinet. The treatments were as follows:

1. Surface sterilized with $HgCl_2$ (0.05-0.3%) for 2 to 6 minutes followed by washing thrice with SDW.
2. Surface sterilized with NaOCl (3-12%) for 10 to 30 minutes followed by washing thrice with SDW.

These treatments were given in order to find out the best surface sterilant for sterilization.

3.2.11 Treatments for phenol exudation

Half strength MS medium was prepared containing different anti-oxidants *viz.*, polyvinyl pyrolidone (PVP)**,** citric acid and ascorbic acid for reducing phenol exudations from the cut surface of the explants:

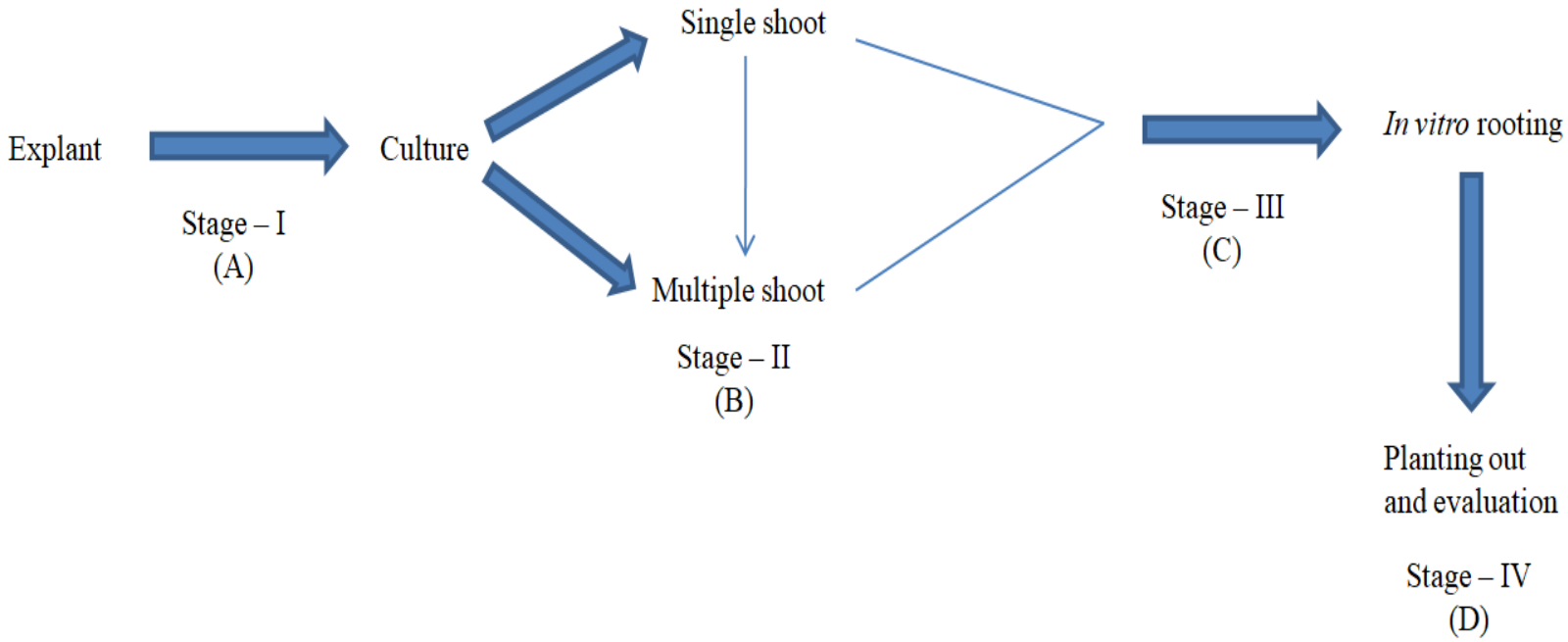
1. The explants were inoculated onto ½ strength MS basal medium containing polyvinyl pyrolidone (2.5 g/l - 10 g/l), citric acid (2.5 g/l - 10 g/l) and ascorbic acid (2.5 g/l - 10 g/l) separately. When the phenols were completely released in the medium, it was discarded and explants were cultured singly on solidified establishment medium.
2. On further exudation of phenols, explants sub-cultured on a fresh medium of same composition till the exudation stopped.
3. Explants were directly cultured on polyvinyl pyrolidone containing establishment medium.
4. Explants were directly cultured on citric acid containing establishment medium.
5. Explants were also directly cultured on ascorbic acid containing establishment medium.

3.2.12 Culture conditions

All the cultures were incubated in a culture room at a temperature of 26 $\pm$ 2 ^{o}C with relative humidity of 55 $\pm$ 5 ^{o}C % and were exposed to 16 hours photoperiod provided by 40-W cool fluorescent tubes and kept at 50 cm above bench surface.

3.2.13 *In vitro* cloning procedure

The general *in vitro* cloning procedure adopted in the present study is presented in figure 3.1.



Stage – I → Culture establishment
Stage – II → Multiplication
Stage - III → *In vitro* rooting
Stage - IV → Planting out and evaluation

Figure 3.1: *In vitro* cloning procedure in clonal apple rootstocks MM106 and MM111

3.2.14 Inoculation and incubation of culture

The culturing of the explants was performed under aseptic conditions in the laminar air flow cabinet. The floor and walls of the cabinet were thoroughly cleaned with cotton dipped in methanol (spirit) just after switching on the laminar air flow cabinet. The floor was again cleaned with methanol just before the inoculation.

Surfaced sterilized explants (shoot tips and nodal segments) were transferred from the bottle to sterile (pre autoclaved) petridishes containing sterile filter paper to absorbs the water adhering to the explants. These explants were inoculated in glass jars and test tubes containing culture medium supplemented with growth regulators at different concentration.

Each glass jar contained 3-5 explants while each test tube contained single explant. The brown end of shoot tips and nodal segments were removed before inoculation to remove the toxic effects of mercuric chloride ($HgCl_2$) and sodium hypochlorite (NaOCl). The explants were inoculated with the help of sterile forceps. After inoculating the explants on to the culture medium, the culture vessels were incubated at 26 $\pm$ 2 ^{o}C temperature in 16 hours continuous fluorescent light (2000 lux) followed by dark period of 8 hours. The data were recorded on the per cent aseptic cultures, per cent survival of cultures and per cent established cultures after six weeks, respectively after inoculation.

3.2.15 Standardization of surface sterilizing agent for *in vitro* propagation of apple clonal rootstocks MM106 and MM111

In order to standardize the most effective surface sterilization method and to isolate contamination free apple rootstocks explants for culture establishment, a trial was conducted using different surface sterilizing agents as given below:

S. No.	Sterilant	Concentration (%)	Duration (Minutes)
1.	Control (Distilled water)	-	-
2.	$HgCl_2$	0.05%	2 minutes
3.	$HgCl_2$	0.05%	4 minutes
4.	$HgCl_2$	0.05%	6 minutes
5.	$HgCl_2$	0.2%	2 minutes
6.	$HgCl_2$	0.2%	4 minutes
7.	$HgCl_2$	0.2%	6 minutes
8.	$HgCl_2$	0.3%	2 minutes
9.	$HgCl_2$	0.3%	4 minutes
10.	$HgCl_2$	0.3%	6 minutes

S. No.	Sterilant	Concentration (%)	Duration (Minutes)
1.	Control (Distilled water)	-	-
2.	NaOCl	3%	10 minutes
3.	NaOCl	8%	20 minutes
4.	NaOCl	12%	30 minutes
5.	NaOCl	3%	10 minutes
6.	NaOCl	8%	20 minutes
7.	NaOCl	12%	30 minutes
8.	NaOCl	3%	10 minutes
9.	NaOCl	8%	20 minutes
10.	NaOCl	12%	30 minutes

The explants after initial treatment with sterilizing agents followed by through washing with distilled water, under aseptic conditions in a laminar air flow cabinet were inoculated on the nutrient medium.

3.2.16 Standardization of media for *in vitro* culture establishment and shoot proliferation in apple clonal rootstocks MM106 and MM111

A. *In vitro* culture establishment

The Murashige and Skoog (1962) medium was used for culture establishment of shoot tip and nodal segment explants of apple clonal rootstocks *viz.,* MM106 and MM111. Different antioxidants and growth regulators were tried to standardize the

most suitable culture establishment medium, the details of which are given as follows:

S. No.	Media code	Treatment
1.	AM1	Control: MS (Basal)
2.	AM2	PVP (2.5 mg/l)
3.	AM3	PVP (5 mg/l)
4.	AM4	PVP (10 mg/l)
5.	AM5	Citric acid (2.5 mg/l)
6.	AM6	Citric acid (5 mg/l)
7.	AM7	Citric acid (10 mg/l)
8.	AM8	Ascorbic acid (2.5 mg/l)
9.	AM9	Ascorbic acid (5 mg/l)
10.	AM10	Ascorbic acid (10 mg/l)

S. No.	Media code	Treatment
1.	EM1	Control: MS (Basal)
2.	EM2	BAP (1.0 mg/l)
3.	EM3	BAP (2.0 mg/l)
4.	EM4	BAP (3.0 mg/l)
5.	EM5	GA_3 (1.0 mg/l)
6.	EM6	GA_3 (2.0 mg/l)
7.	EM7	GA_3 (3.0 mg/l)
8.	EM8	BAP (1.0 mg/l) + GA_3 (1.0 mg/l)
9.	EM9	BAP (1.0 mg/l) + GA_3 (2.0 mg/l)
10.	EM10	BAP (1.0 mg/l) + GA_3 (3.0 mg/l)
11.	EM11	BAP (2.0 mg/l) + GA_3 (1.0 mg/l)
12.	EM12	BAP (2.0 mg/l) + GA_3 (2.0 mg/l)
13.	EM13	BAP (2.0 mg/l) + GA_3 (3.0 mg/l)
14.	EM14	BAP (3.0 mg/l) + GA_3 (1.0 mg/l)
15.	EM15	BAP (3.0 mg/l) + GA_3 (2.0 mg/l)
16.	EM16	BAP (3.0 mg/l) + GA_3 (3.0 mg/l)

B. *In vitro* shoot proliferation

The *in vitro* shoots obtained by culturing the explants on establishment medium, served as a material for proliferation. After four to six weeks, the sprouted segments were taken out aseptically with the help of sterilized forceps from culture vessels. The optimum concentration and combination of the plant growth regulators for *in vitro* shoot proliferation was standardized. The observations on number of days taken for shoot proliferation, number of shoots per explants, length of the longest shoot (cm) and number of leaves per explants were recorded after three to four sub-cultures and culture duration was of one to two weeks, depending upon the culture status.

S. No.	Media code	Treatments
1.	MM1	Control: MS (Basal)
2.	MM2	BAP (1.0 mg/l)
3.	MM3	BAP (2.0 mg/l)
4.	MM4	BAP (3.0 mg/l)
5.	MM5	GA_3 (1.0 mg/l)
6.	MM6	GA_3 (2.0 mg/l)
7.	MM7	GA_3 (3.0 mg/l)
8.	MM8	BAP (1.0 mg/l) + GA_3 (1.0 mg/l)
9.	MM9	BAP (1.0 mg/l) + GA_3 (2.0 mg/l)
10.	MM10	BAP (1.0 mg/l) + GA_3 (3.0 mg/l)
11.	MM11	BAP (2.0 mg/l) + GA_3 (1.0 mg/l)
12.	MM12	BAP (2.0 mg/l) + GA_3 (2.0 mg/l)
13.	MM13	BAP (2.0 mg/l) + GA_3 (3.0 mg/l)
14.	MM14	BAP (3.0 mg/l) + GA_3 (1.0 mg/l)
15.	MM15	BAP (3.0 mg/l) + GA_3 (2.0 mg/l)
16.	MM16	BAP (3.0 mg/l) + GA_3 (3.0 mg/l)

* NAA @ 0.2% was used in each treatment

3.2.17 Standardization of rooting medium for *in vitro* propagation of clonal apple rootstocks MM106 and MM111

Half strength MS medium supplemented with growth hormones was tried for rooting in plantlets of clonal apple rootstocks MM106 and MM111 raised *in vitro*, and finally the effect of media on *in vitro* rooting of plantlets with better performing auxins like IBA and NAA at different concentrations were studied. The per cent culture or plantlets rooted, numbers of days taken for root initiation, number of roots per plants and length of the longest root (cm) were recorded after six weeks of inoculation.

S. No.	Media code	Treatments
1.	RM1	Control: MS (Basal)
2.	RM2	IBA (0.5 mg/l)
3.	RM3	IBA (1.0 mg/l)
4.	RM4	IBA (2.0 mg/l)
5.	RM5	NAA (0.5 mg/l)
6.	RM6	NAA (1.0 mg/l)
7.	RM7	NAA (2.0 mg/l)
8.	RM8	IBA (0.5 mg/l) + NAA (0.5 mg/l)
9.	RM9	IBA (0.5 mg/l)+ NAA (1.0 mg/l)
10.	RM10	IBA (0.5 mg/l) + NAA (2.0 mg/l)
11.	RM11	IBA (1.0 mg/l) + NAA (0.5 mg/l)
12.	RM12	IBA (1.0 mg/l) + NAA (1.0 mg/l)
13.	RM13	IBA (1.0 mg/l) + NAA (2.0 mg/l)
14.	RM14	IBA (2.0 mg/l) + NAA (0.5 mg/l)
15.	RM15	IBA (2.0 mg/l) + NAA (1.0 mg/l)
16.	RM16	IBA (2.0 mg/l) + NAA (2.0 mg/l)

*Activated charcoal @ 0.2% was used in each treatment

3.2.18 Standardization of soil medium for *in vitro* raised plantlets of apple clonal rootstocks MM106 and MM111

In order to acclimatize the *in vitro* produced clonal apple rootstocks plantlets, a study on different hardening treatment was carried out. Precaution was taken to

avoid any possible damage to delicate root system of the plant. Uniformly rooted plantlets were taken from the culture vessels with the help of forceps. The agar medium attached to the roots was washed gently under the running tap water. After removing of adhering medium, the plants were transplanted, 6 weeks after root initiation, into pots filled with media containing sand, soil, vermiculite and FYM alone and in combination. Then they were subjected to hardening by covering the plants with polythene bag/ glass beaker and maintaining humidity by providing mist spray of water over the plants by removing the cover while keeping in the culture room from time to time.

The plantlets were covered with polythene bag/ glass beaker for 3 weeks in all treatments. For initial 3 days no air was allowed to enter and then air space provided in the cover. In all treatment, the plantlets were kept in continuous light (approx. 800 lux) in the culture room at a temperature of 26 $\pm$ 2 ^{o}C. The cover was gradually removed after 6 days, initially for 3 hours followed by 6 hour and 12 hours in next 6 days. Subsequently, the period of keeping the plantlets at room temperature was gradually increased or increasing holes in the polythene bags with time, after 3 weeks were brought outside A/C room. All the plantlets were watered through sprayer every day.

Potting mixtures were made up of different ingredients as detailed below were tried:

S. No.	Media code	Treatments
1.	SM1	Sand (100%)
2.	SM2	Soil (100%)
3.	SM3	Vermiculite (100%)
4.	SM4	FYM (100%)
5.	SM5	Soil + Sand + Vermiculite (1:1:1 v/v/v)
6.	SM6	Soil + Sand + FYM (1:1:1 v/v/v)
7.	SM7	Soil + Sand + Vermiculite + FYM (1:1:1:1 v/v/v/v)

3.2.19 Economic analysis of propagation of apple clonal rootstocks MM106 and MM111

The cost of production of two clonal apple rootstocks MM106 and MM111 using the technological information as per the protocol developed in the present investigation was computed based on the facilities available and based on the

percentage of survival, rate of culture establishment, rate of proliferation, rooting response of shoots, *ex vitro* survival of the plant, maximum capacity of the laboratory and the number of initial explant capable of producing maximum plantlets was calculated. The total cost involved per cycle was worked out, which involves the cost of building, equipments, glassware, chemicals and miscellaneous items having been distributed over the years according to their potential durability.

3.3 Statistical analysis:

The data generated from the different experiments were subjected to statistical analysis in completely randomized design (CRD) as described by Singh *et al*. (1998).

Results

CHAPTER - IV

RESULTS

The results of the present investigation entitled "Propagation studies on clonal rootstocks of apple (*Malus* × *domestica* Borkh.)" conduced at Plant Tissue Culture Laboratory of School of Biotechnology and Tissue Culture Laboratory, Advanced Centre for Horticulture Research (ACHR), Udheyawalla of Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu, Chatha during the year 2018 are presented in this chapter under the following heads:

4.1 Effect of mercuric chloride ($HgCl_2$) and sodium hypochlorite (NaOCl) on sterilization of explants of clonal apple rootstocks MM106 and MM111

Before surface sterilization of explants of apple clonal rootstocks with $HgCl_2$ or NaOCl, the shoot tip and nodal segment of MM106 and MM111 were given a treatment with Bavistin (0.2%) for 20 minutes. The surface sterilants mercuric chloride ($HgCl_2$) and sodium hypochlorite (NaOCl) in different concentration and duration were used. The data on per cent aseptic culture and per cent survival under mercuric chloride (0.05% to 0.2%) and sodium hypochlorite (3% to 12%) for different duration (2 to 6 minutes and 10 to 30 minutes, respectively) and during different months (March, April and May) are presented in table 4.1.1, 4.1.2, 4.2.1, 4.2.2, 4.3.1, 4.3.2, 4.4.1, 4.4.2, 4.5.1, 4.5.2, 4.6.1 and 4.6.2 and figure 4.1, 4.2, 4.3, 4.4, 4.5, 4.6, 4.7, 4.8, 4.9, 4.10, 4.11 and 4.12.

4.1.1 Effect of mercuric chloride ($HgCl_2$) on per cent aseptic cultures in apple clonal rootstocks during March 2018

The data presented in table 4.1.1 and figure 4.1 showed that different treatments of mercuric chloride ($HgCl_2$) had a significant influence on per cent aseptic cultures of clonal apple rootstocks MM106 and MM111 during the month of March 2018. The data with respect to per cent aseptic cultures of clonal apple rootstock MM106 revealed that the maximum aseptic cultures (68.33%) were recorded with 0.1% $HgCl_2$ for 4 minutes which was significantly superior over all other treatments and was followed by 0.1% $HgCl_2$ for 6 minutes (61.67%), 0.1% $HgCl_2$ for 2 minutes (53.33%) and 0.2% $HgCl_2$ for 2 minutes (45.00%), whereas the minimum aseptic cultures (0.00%) were recorded under control (without sterilizing

agents). Between the explants, nodal segment explants gave maximum aseptic cultures (39.33%) which were significantly higher than the shoot tip (29.67%). In the interaction effect, 0.1% $HgCl_2$ for 4 minutes gave maximum aseptic cultures (73.33%) followed by 66.67 per cent with 0.1% $HgCl_2$ for 6 minutes in nodal segment, while maximum aseptic cultures (63.33%) in shoot tip were recorded with 0.1% $HgCl_2$ for 4 minutes followed by 0.1% $HgCl_2$ for 6 minutes (56.67%). However, in both the explants, minimum aseptic cultures (0.00%) were recorded under control.

In MM111 rootstock, maximum aseptic cultures (71.67%) were observed with 0.1% $HgCl_2$ for 4 minutes which was significantly higher over rest of the treatments and was followed by 0.1% $HgCl_2$ for 6 minutes (65.00%), 0.1% $HgCl_2$ for 2 minutes (56.67%) and 0.2% $HgCl_2$ for 2 minutes (48.33%), whereas the minimum aseptic cultures (0.00%) were recorded under control. Between the explants, maximum aseptic cultures (42.33%) were observed in nodal segment explants which were significantly higher than the shoot tip (32.67%). The interaction data revealed that nodal segment explants under 0.1% $HgCl_2$ for 4 minutes treatment gave maximum aseptic cultures (76.67%) followed by 70.00 per cent with 0.1% $HgCl_2$ for 6 minutes, while shoot tip recorded maximum aseptic cultures (66.67%) with 0.1% $HgCl_2$ for 4 minutes followed by 0.1% $HgCl_2$ for 6 minutes (60.00%). However, in both the explants, minimum aseptic cultures (0.00%) were recorded under control.

4.1.2 Effect of sodium hypochlorite (NaOCl) on per cent aseptic cultures in apple clonal rootstocks during March 2018

The data enumerated in table 4.1.2 and figure 4.2 revealed that different treatments of sodium hypochlorite (NaOCl) significantly influenced the per cent aseptic cultures in clonal apple rootstocks MM106 and MM111 during the month of March 2018. The clonal apple rootstock MM106 recorded maximum aseptic cultures (63.33%) with 8% NaOCl for 20 minutes which was significantly higher over rest of the treatments and was followed by 8% NaOCl for 30 minutes (56.67%) and 12% NaOCl for 10 minutes (50.00%), whereas the minimum aseptic cultures (0.00%) were recorded under control (without sterilizing agents). Between the explants (shoot tip and nodal explants), nodal explants gave maximum aseptic cultures (38.00%) which were significantly superior to the shoot tip (30.33%). The interaction effect with respect to per cent aseptic cultures revealed that maximum aseptic cultures (66.67%)

Table 4.1.1: Effect of mercuric chloride ($HgCl_2$) on per cent aseptic cultures in apple clonal rootstocks during March 2018

Treatment	Duration (min.)	MM106			MM111		
		Shoot tip	Nodal segment	Mean	Shoot tip	Nodal segment	Mean
Control	-	0.00 (0.00)*	0.0 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
$HgCl_2$ (0.05%)	2	10.00 (18.42)	26.67 (30.98)	18.33 (24.70)	13.33 (26.55)	30.00 (35.20)	21.67 (30.87)
$HgCl_2$ (0.05%)	4	16.67 (23.84)	30.00 (33.19)	23.33 (28.52)	20.00 (30.98)	33.33 (39.21)	26.67 (35.09)
$HgCl_2$(0.05%)	6	16.67 (23.84)	33.33 (35.20)	25.00 (29.52)	20.00 (30.98)	36.67 (39.21)	28.33 (35.09)
$HgCl_2$ (0.1%)	2	50.00 (44.98)	56.67 (48.82)	53.33 (46.90)	53.33 (50.74)	60.00 (50.74)	56.67 (50.74)
$HgCl_2$ (0.1%)	4	63.33 (52.75)	73.33 (58.98)	68.33 (55.86)	66.67 (58.98)	76.67 (63.40)	71.67 (61.19)
$HgCl_2$ (0.1%)	6	56.67 (48.82)	66.67 (54.76)	61.67 (51.79)	60.00 (54.76)	70.00 (58.98)	65.00 (56.87)
$HgCl_2$ (0.2%)	2	43.33 (41.13)	46.67 (43.06)	45.00 (42.09)	46.67 (46.90)	50.00 (48.82)	48.33 (41.86)
$HgCl_2$ (0.2%)	4	23.33 (28.76)	36.67 (37.21)	30.00 (32.98)	26.67 (35.20)	40.00 (43.06)	33.33 (39.13)
$HgCl_2$ (0.2%)	6	16.67 (24.03)	23.33 (28.76)	20.00 (26.40)	20.00 (30.98)	26.67 (37.21)	23.33 (34.09)
Mean		29.67 (30.66)	39.33 (37.09)		32.67 (36.61)	42.33 (41.58)	
$CD_{(0.05)}$	Explant (E) = 1.66 Sterilant (S) = 3.71 E × S = 5.24				Explant (E) = 1.45 Sterilant (S) = 3.26 E × S = 4.61		

*Figures in parenthesis are arcsine transformed values

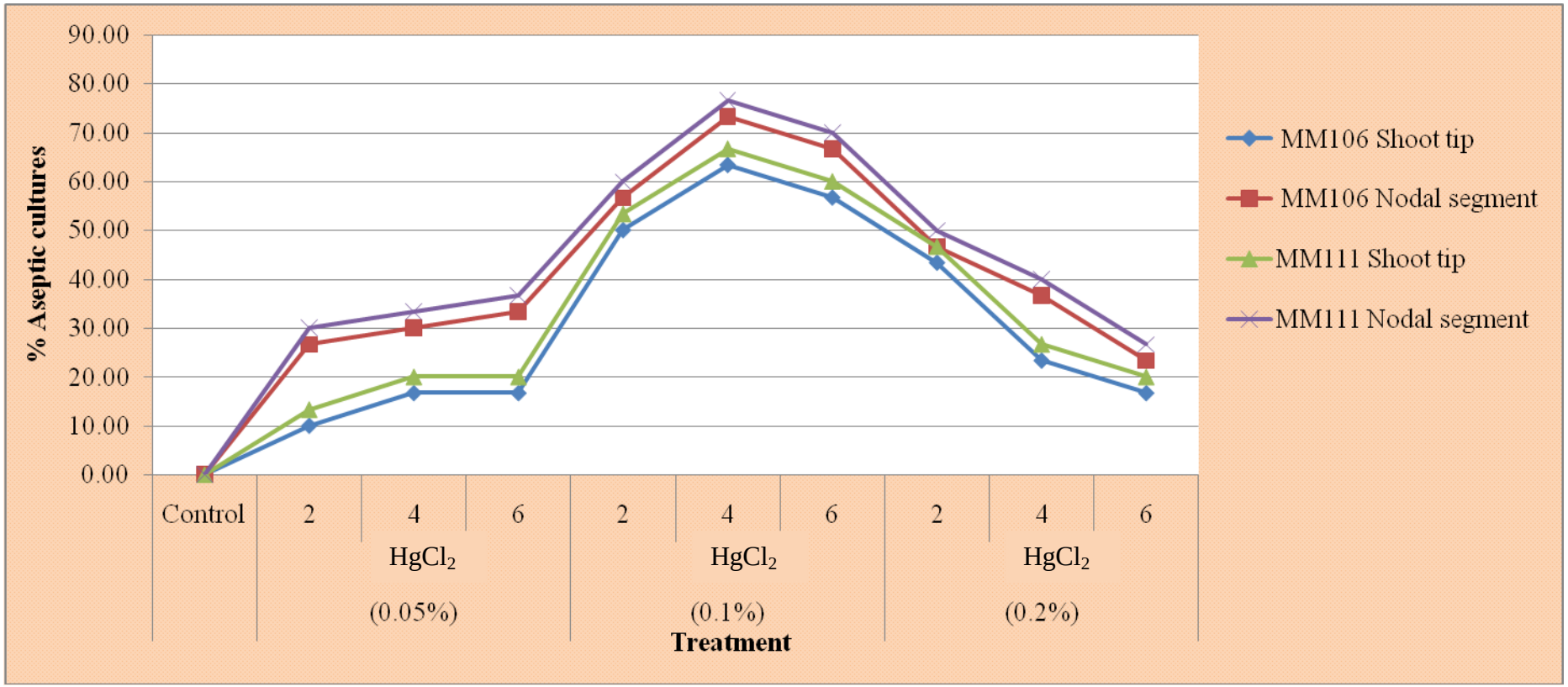


Figure 4.1: Effect of mercuric chloride ($HgCl_2$) on per cent aseptic cultures of clonal apple rootstocks during March 2018

Table 4.1.2: Effect of sodium hypochlorite (NaOCl) on per cent aseptic cultures in apple clonal rootstocks during March 2018

Treatment	Duration (min.)	MM106			MM111		
		Shoot tip	Nodal segment	Mean	Shoot tip	Nodal segment	Mean
Control	-	0.00 (0.00)*	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
NaOCl (3%)	**10**	13.33 (21.13)	23.33 (28.76)	18.33 (24.95)	16.67 (23.84)	26.67 (30.98)	21.67 (27.41)
NaOCl (3%)	**20**	20.00 (26.55)	33.33 (35.20)	26.67 (30.87)	20.00 (26.55	33.33 (35.20)	26.67 (30.87)
NaOCl (3%)	**30**	20.00 (26.55)	36.67 (37.21)	28.33 (31.88)	20.00 (26.55)	36.67 (37.21)	28.33 (31.88)
NaOCl (8%)	**10**	33.33 (35.20)	46.67 (43.06)	40.00 (39.13)	33.33 (35.20	46.67 (43.06)	40.00 (39.13)
NaOCl (8%)	**20**	(60.00 (50.74)	66.67 (54.76)	63.33 (52.75)	63.33 (52.75)	70.00 (56.76)	66.67 (54.76)
NaOCl (8%)	**30**	56.67 (48.82)	56.67 (48.82)	56.67 (48.82)	60.00 (50.83)	60.00 (50.74)	60.00 (50.79)
NaOCl (12%)	**10**	46.67 (43.06)	53.33 (46.90)	50.00 (44.98)	46.67 (43.06)	56.67 (48.82)	51.67 (45.94)
NaOCl (12%)	**20**	33.33 (35.20)	46.67 (43.06)	40.00 (39.13)	33.33 (35.20)	46.67 (43.06)	40.00 (39.13)
NaOCl (12%)	**30**	20.00 (26.55)	16.67 (23.84)	18.33 (25.20)	20.00 (26.55)	16.67 (23.84)	18.33 (25.20)
Mean		30.33 (31.38)	38.00 (36.16)		31.33 (32.05)	39.33 (36.97)	
$CD_{(0.05)}$	Explant (E) = 1.59 Sterilant (S) = 3.56 E × S = 5.04				Explant (E) = 1.64 Sterilant (S) = 3.68 E × S = 5.21		

*Figures in parenthesis are arcsine transformed values

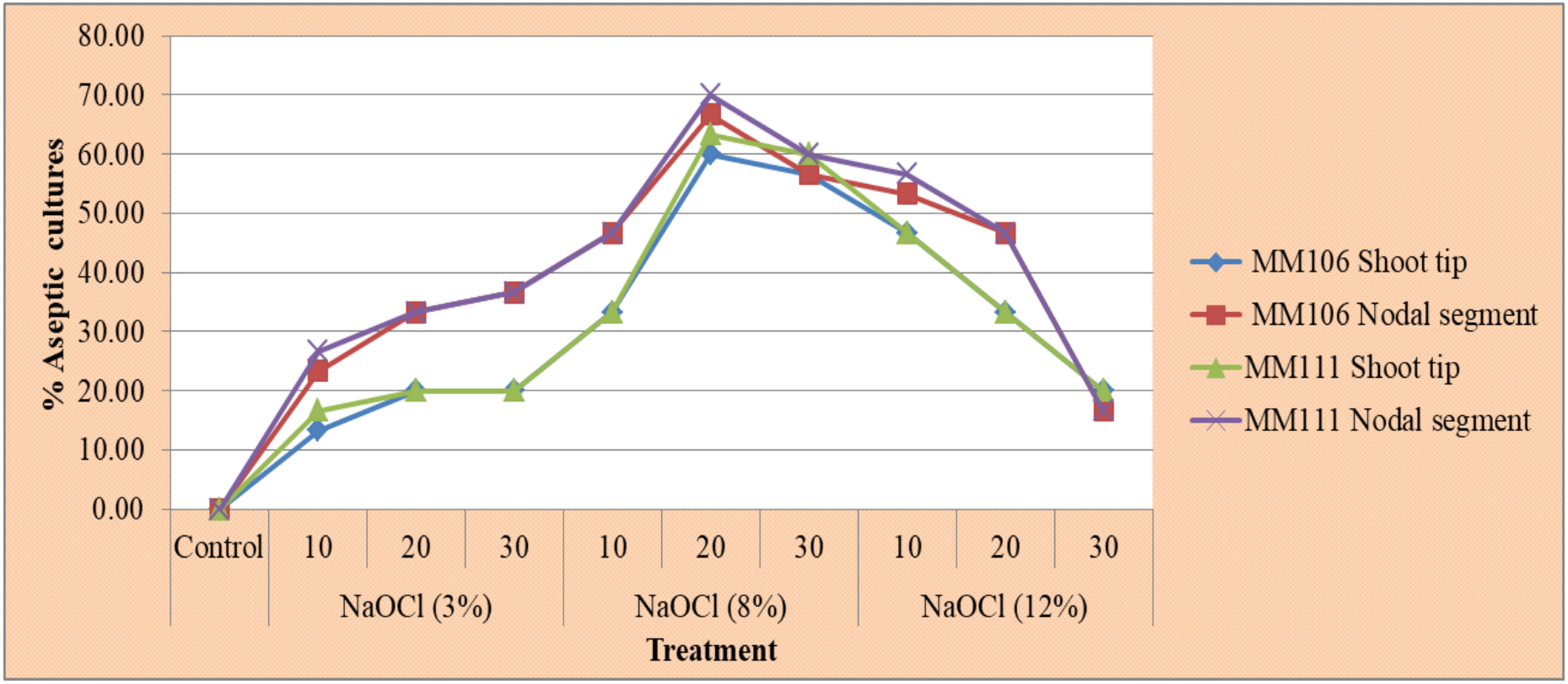


Figure 4.2: Effect of sodium chloride (NaOCl) on per cent aseptic cultures of clonal apple rootstocks during March 2018

Table 4.2.1: Effect of mercuric chloride ($HgCl_2$) on per cent aseptic cultures in apple clonal rootstocks during April 2018

Treatment	Duration (min.)	MM106			MM 111		
		Shoot tip	Nodal segment	Mean	Shoot tip	Nodal segment	Mean
Control	-	0.00 (0.00)*	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
$HgCl_2$ (0.05%)	2	26.67 (30.98)	40.00 (39.21)	33.33 (35.09)	26.67 (30.98)	40.00 (39.21)	33.33 (35.09)
$HgCl_2$ (0.05%)	4	30.00 (33.19)	40.00 (39.21)	35.00 (36.20)	30.00 (33.19)	43.33 (41.13)	36.67 (37.16)
$HgCl_2$ (0.05%)	6	30.00 (33.19)	43.33 (41.13)	36.67 (37.16)	33.33 (35.20)	46.67 (43.06)	40.00 (39.13)
$HgCl_2$(0.1%)	2	63.33 (52.75)	63.33 (52.75)	63.33 (52.75)	63.33 (52.75)	63.33 (52.75)	63.33 (52.75)
$HgCl_2$ (0.1%)	4	80.00 (63.40)	86.67 (68.82)	83.33 (66.11)	80.00 (63.40)	86.67 (68.82)	83.33 (66.11)
$HgCl_2$ (0.1%)	6	66.67 (54.76)	73.33 (58.98)	70.00 (56.87)	70.00 (56.76)	76.67 (66.19)	73.33 (58.98)
$HgCl_2$ (0.2%)	2	53.33 (46.90)	56.67 (48.82)	55.00 (47.86)	56.67 (48.82)	60.00 (50.75)	58.33 (49.78)
$HgCl_2$ (0.2%)	4	33.33 (35.20)	50.00 (44.98)	41.67 (40.09)	36.67 (37.21)	50.00 (44.98)	43.33 (41.09)
$HgCl_2$ (0.2%)	6	26.67 (30.98)	36.67 (37.21)	31.67 (34.09)	30.00 (33.19)	40.00 (39.21)	35.00 (36.20)
Mean		41.00 (38.13)	49.00 (43.11)		42.67 (39.15)	51.85 (44.41)	
$CD_{(0.05)}$	Explant (E) = 1.47 Sterilant (S) = 3.29 E × S = 4.66				Explant (E) = 1.34 Sterilant (S) = 3.00 E × S = 4.26		

*Figures in parenthesis are arcsine transformed values

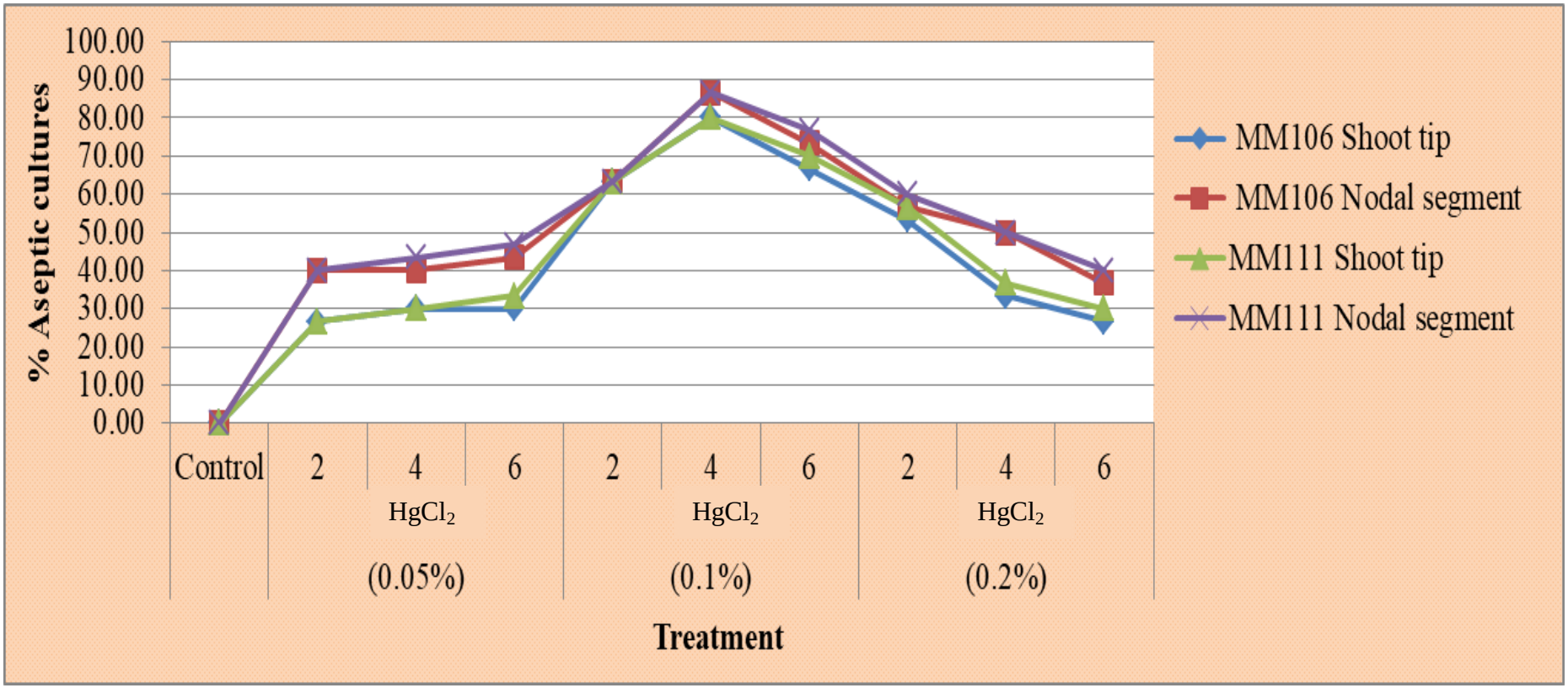


Figure 4.3: Effect of mercuric chloride ($HgCl_2$) on per cent aseptic cultures of clonal apple rootstocks during April 2018

Table 4.2.2: Effect of sodium hypochlorite (NaOCl) on per cent aseptic cultures in apple clonal rootstocks during April 2018

Treatment	Duration (min.)	MM106			MM111		
		Shoot tip	Nodal segment	Mean	Shoot tip	Nodal segment	Mean
Control	-	0.00 (0.00)*	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
NaOCl (3%)	**10**	23.33 (28.76)	30.00 (30.87)	26.67 (30.87)	26.67 (30.98)	33.33 (35.20)	30.00 (33.09)
NaOCl (3%)	**20**	30.00 (33.19)	43.33 (37.16)	36.67 (37.16)	33.33 (35.20)	46.67 (43.06)	40.00 (39.13)
NaOCl (3%)	**30**	30.00 (33.19)	53.33 (40.05)	41.67 (40.05)	33.33 (35.20)	56.67 (48.82)	45.00 (42.01)
NaOCl (8%)	**10**	43.33 ‘(41.13)	60.00 (45.98)	51.67 (45.98)	46.67 (43.06)	60.00 (50.74)	53.33 (46.90)
NaOCl (8%)	**20**	63.33 (52.75)	76.67 (56.97)	70.00 (56.97)	66.67 (54.76)	80.00 (63.40)	73.33 (59.08)
NaOCl (8%)	**30**	63.33 (52.75)	66.67 (53.75)	65.00 (53.75)	63.33 (52.75)	70.00 (56.76)	66.67 (54.76)
NaOCl (12%)	**10**	60.00 (50.83)	60.00 (50.79)	60.00 (50.79)	66.67 (54.76)	66.67 (54.76)	66.67 (54.76)
NaOCl (12%)	**20**	53.33 (46.90)	53.33 (46.90)	53.33 (46.90)	53.33 (46.90)	56.67 (48.82)	55.00 (47.86)
NaOCl (12%)	**30**	20.00 (26.06)	33.33 (30.63)	26.67 (30.63)	26.67 (30.98)	33.33 (35.20)	30.00 (33.09)
Mean		38.67 (36.56)	47.67 (42.06)		41.67 (38.46)	50.33 (43.68)	
$CD_{(0.05)}$	Explant (E) = 2.02 Sterilant (S) = 4.51 E × S = 6.38				Explant (E) = 1.72 Sterilant (S) = 3.84 E × S = 5.43		

*Figures in parenthesis are arcsine transformed values

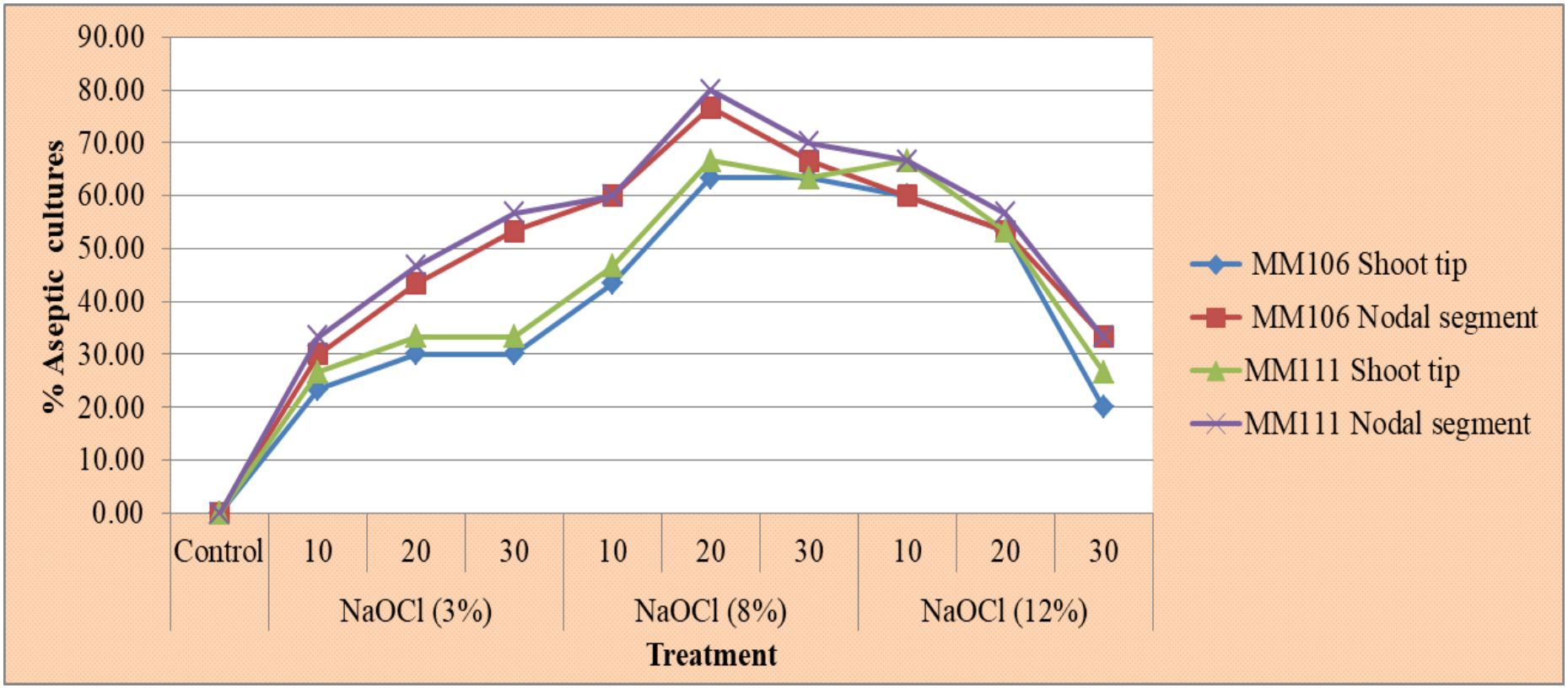


Figure 4.4: Effect of sodium chloride (NaOCl) on per cent aseptic cultures of clonal apple rootstocks during April 2018

Table 4.3.1: Effect of mercuric chloride ($HgCl_2$) on per cent aseptic cultures in apple clonal rootstocks during May 2018

Treatment	Duration (min.)	MM106			MM111		
		Shoot tip	Nodal segment	Mean	Shoot tip	Nodal segment	Mean
Control	-	0.00 (0.00)*	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
$HgCl_2$ (0.05%)	2	16.67 (24.06)	30.00 (33.19)	23.33 (28.61)	20.00 (26.55)	33.33 (35.20)	26.67 (30.87)
$HgCl_2$ (0.05%)	4	23.33 (28.76)	36.67 (37.21)	30.00 (32.98)	26.67 (30.98)	40.00 (39.21)	33.33 (35.09)
$HgCl_2$(0.05%)	6	23.33 (28.76)	33.33 (35.20)	28.33 (31.98)	26.67 (30.98)	36.67 (37.21)	31.67 (34.09)
$HgCl_2$ (0.1%)	2	56.67 (48.82)	56.67 (48.82)	56.67 (48.82)	60.00 (50.74)	60.00 (50.74)	60.00 (50.74)
$HgCl_2$ (0.1%)	4	70.00 (56.76)	76.67 (61.19)	73.33 (58.98)	73.33 (58.98)	80.00 (63.40)	76.67 (61.19)
$HgCl_2$ (0.1%)	6	63.33 (52.75)	70.00 (56.76)	66.67 (54.76)	66.67 (54.76)	73.33 (58.98)	70.00 (56.87)
$HgCl_2$ (0.2%)	2	50.00 (44.98)	53.33 (46.90)	51.67 (45.94)	53.33 (46.90)	56.67 (48.82)	55.00 (47.86)
$HgCl_2$ (0.2%)	4	30.00 (33.19)	43.33 (41.13)	36.67 (37.16)	33.33 (35.20)	46.67 (43.06)	40.00 (39.13)
$HgCl_2$(0.2%)	6	23.33 (28.76)	36.67 (37.21)	30.00 (32.98)	26.67 (30.98)	40.00 (39.21)	33.33 (35.09)
Mean		35.67 (34.68)	43.67 (39.76)		39.00 (36.61)	47.00 (41.58)	
$CD_{(0.05)}$	Explant (E) = 1.46 Sterilant (S) = 3.27 E × S = 4.63				Explant (E) = 1.03 Sterilant (S) = 2.31 E × S = 3.25		

*Figures in parenthesis are arcsine transformed values

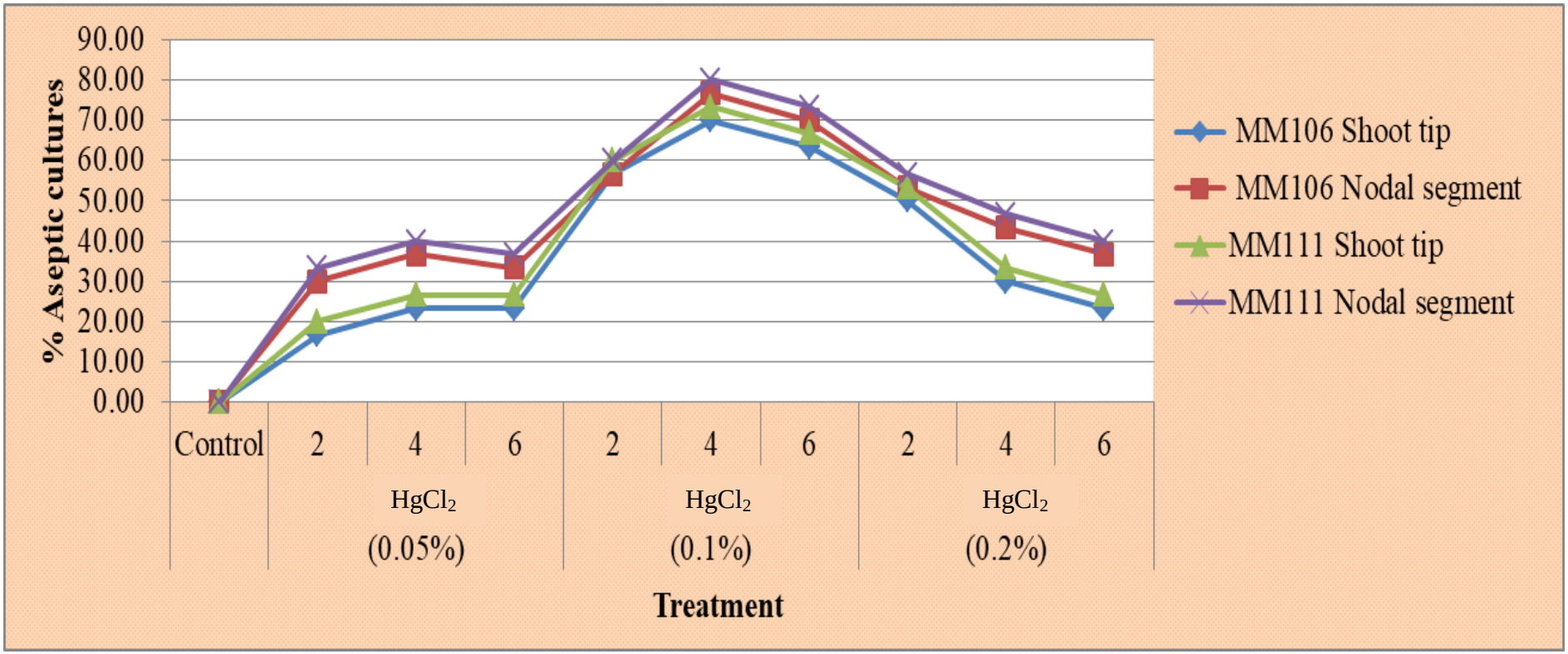


Figure 4.5: Effect of mercuric chloride ($HgCl_2$) on per cent aseptic cultures of clonal apple rootstocks during May 2018

Table 4.3.2: Effect of sodium hypochlorite (NaOCl) on per cent aseptic cultures in apple clonal rootstocks during May 2018

Treatment	Duration (min.)	MM106			MM111		
		Shoot tip	Nodal segment	Mean	Shoot tip	Nodal segment	Mean
Control	-	0.00 (0.00)*	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
NaOCl (3%)	**10**	20.00 (26.55)	26.67 (30.98)	23.33 (28.76)	20.00 (26.55)	30.00 (33.19)	25.00 (29.87)
NaOCl (3%)	**20**	23.33 (28.76)	40.00 (39.21)	31.67 (33.09)	26.67 (30.98)	40.00 (39.21)	33.33 (35.09)
NaOCl (3%)	**30**	26.67 (30.98)	43.33 (41.13)	35.00 (36.06)	26.67 (30.98)	46.67 (43.06)	36.67 (37.02)
NaOCl (8%)	**10**	53.33 (46.90)	60.00 (50.74)	56.67 (48.82)	56.67 (48.82)	60.00 (50.74)	58.33 (49.78)
NaOCl (8%)	**20**	60.00 (50.74)	70.00 (56.76)	65.00 (53.75)	60.00 (50.74)	73.33 (58.98)	66.67 (54.86)
NaOCl (8%)	**30**	56.67 (48..82)	63.33 (52.75)	60.00 (50.79)	56.67 (48.82)	63.33 (52.75)	60.00 (50.79)
NaOCl (12%)	**10**	46.67 (43.06)	53.33 (46.90)	50.00 (44.98)	46.67 (43.06)	50.00 (44.98)	48.33 (44.02)
NaOCl (12%)	**20**	40.00 (39.21)	43.33 (49.13)	41.67 (40.17)	40.00 (39.21)	53.33 (46.90)	46.67 (43.06)
NaOCl (12%)	**30**	20.00 (26.55)	26.67 (30.98)	23.33 (28.76)	20.00 (26.55)	26.67 (30.98)	23.33 (28.76)
Mean		34.67 (34.16)	42.67 (39.06)		35.33 (34.57)	44.33 (40.08)	
$CD_{(0.05)}$	Explant (E) = 1.37 Sterilant (S) = 3.07 E × S = 4.34			Explant (E) = 1.48 Sterilant (S) = 3.31 E × S = 4.68			

*Figures in parenthesis are arcsine transformed values

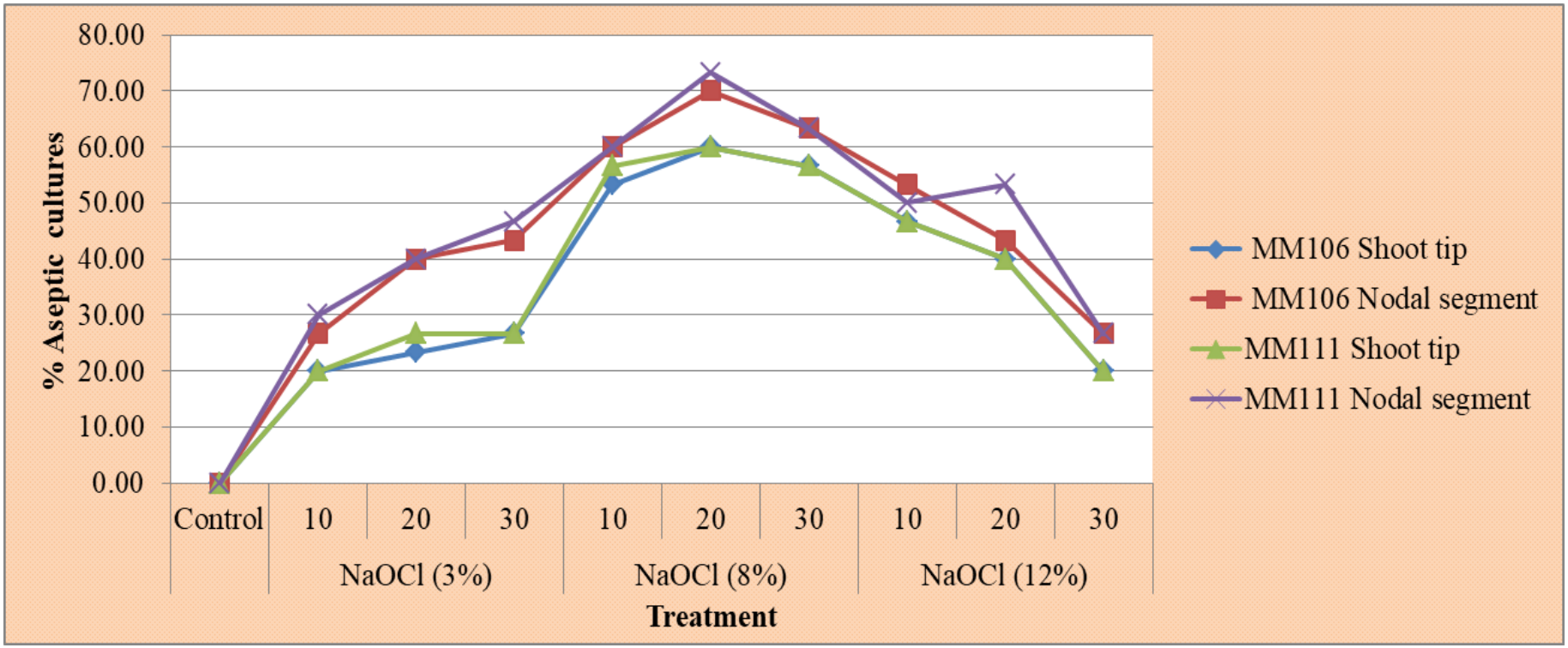


Figure 4.6: Effect of sodium chloride (NaOCl) on per cent aseptic culture of clonal apple rootstocks during May 2018

in nodal segment explants was observed with 8% NaOCl for 20 minutes followed by 8% NaOCl for 30 minutes (56.67%), while maximum aseptic cultures in shoot tip (60.00%) were recorded with 8% NaOCl for 20 minutes followed by 8% NaOCl for 30 minutes (56.67%). However, in both the explants, minimum aseptic cultures (0.00%) were recorded under control.

In clonal apple rootstock MM111, maximum aseptic cultures (66.67%) were observed with 8% NaOCl for 20 minutes which was significantly superior over rest of the treatments and was followed by 8% NaOCl for 30 minutes (68.00%) and 12% NaOCl for 10 minutes (51.67%), whereas the minimum aseptic cultures (0.00%) were recorded under control. Between the explants, nodal segment explants gave maximum aseptic cultures (39.33%) which were significantly higher than the shoot tip (31.33%). In the interaction effect, nodal segment explants under 8% NaOCl for 20 minutes gave maximum aseptic cultures (70.00%) followed by 8% NaOCl for 30 minutes (60.00%), while maximum aseptic cultures (63.33%) in shoot tip were recorded with 8% NaOCl for 20 minutes followed by 8% NaOCl for 30 minutes (60.00%). However, in both the explants, minimum aseptic cultures (0.00%) were recorded in control.

4.2.1 Effect of mercuric chloride ($HgCl_2$) on per cent aseptic cultures in apple clonal rootstocks during April 2018

The data inferred in table 4.2.1 and figure 4.3 revealed that different treatments of mercuric chloride ($HgCl_2$) showed a significant influence on per cent aseptic cultures in clonal apple rootstock MM106 and MM111 during the month of April 2018. In MM106 rootstock, 0.1% $HgCl_2$ for 4 minutes gave maximum aseptic cultures (83.33%) which were significantly higher over rest of the treatments and was followed by 0.1% $HgCl_2$ for 6 minutes (70.00%), 0.1% $HgCl_2$ for 2 minutes (63.33%) and 0.2% $HgCl_2$ for 2 minutes (55.00%), whereas the minimum aseptic culture (0.00%) were recorded under control (without sterilizing agents). Between the two explants, nodal segment gave 49.00 per cent aseptic cultures which were significantly higher than the shoot tip (41.00%). The interaction data revealed that nodal segment explants with 0.1% $HgCl_2$ for 4 minutes gave maximum aseptic cultures (86.67%) followed by 73.33 per cent with 0.1% $HgCl_2$ for 6 minutes, while shoot tip recorded 80.00 per cent aseptic cultures followed by 66.67 per cent with 0.1 $HgCl_2$for 6

minutes. However, minimum aseptic cultures (0.00%) for both the explants were recorded under control.

In clonal apple rootstock MM111, 0.1% $HgCl_2$ for 4 minutes gave maximum aseptic cultures (83.33%) which was significantly superior among all other treatments and was followed by 0.1% $HgCl_2$ for 6 minutes (73.33%), 0.1% $HgCl_2$ for 2 minutes (63.33%) and 0.2% $HgCl_2$ for 2 minutes (58.33%), whereas the minimum aseptic cultures (0.00%) were recorded under control. Between the explants, nodal segment gave maximum average value (51.85%) for aseptic cultures which was significantly superior to shoot tip (42.67%). The interaction data indicated that nodal segment explants treated with 0.1% $HgCl_2$ for 4 minutes gave maximum aseptic cultures (86.67%) followed by 0.1% $HgCl_2$ for 6 minutes (76.67%), while shoot tips recorded 80.00 per cent with 0.1% $HgCl_2$ for 4 minutes followed by 0.1% $HgCl_2$ for 6 minutes (70.00%). However, minimum aseptic cultures (0.00%) were recorded under control for both the explants.

4.2.2 Effect of sodium hypochlorite (NaOCl) on per cent aseptic cultures in apple clonal rootstocks during April 2018

The per cent aseptic culture with respect to clonal apple rootstock MM106 and MM111 during the month of April 2018 is presented in table 4.2.2 and figure 4.4. In MM106 rootstock, maximum aseptic cultures (70.00%) were obtained with 8% NaOCl for 20 minutes which was significantly superior over rest of the treatments and was followed by 8% NaOCl for 30 minutes (65.00%) and 12% NaOCl for 10 minutes (60.00%), while minimum per cent aseptic cultures (0.00%) were obtained under control (without sterilizing agent). Between the explants, nodal segment explants gave maximum aseptic cultures (47.67%) which were significantly higher than the shoot tip (38.67%). In the interaction effect, nodal segment explants with 8% NaOCl for 20 minutes gave maximum aseptic cultures (76.67%) followed by 8% NaOCl for 30 minutes (66.67%), whereas in shoot tip, maximum aseptic cultures (63.33%) were obtained with 8% NaOCl for 20 minutes as well as 8% NaOCl for 30 minutes followed by 12% NaOCl for 10 minutes (60.00%). However, minimum aseptic cultures (0.00%) for both the explants were recorded under control.

The surface sterilization of clonal apple rootstock MM111 with NaOCl also showed a significant effect on per cent aseptic cultures. Maximum aseptic cultures

(73.33%) were obtained with 8% NaOCl for 20 minutes which was significantly higher over rest of the treatments and was followed by 66.67 per cent with 8% NaOCl for 30 minutes as well as 12% NaOCl for 10 minutes, whereas the minimum aseptic cultures (0.00%) were recorded under control. Between the explants, nodal segments gave maximum aseptic cultures (50.33%) which were significantly higher than the shoot tip (41.67%). In the interaction effect, nodal segment explants under 8% NaOCl for 20 minutes treatment gave maximum aseptic cultures (80.00%) followed by 8% NaOCl for 30 minutes (70.00%), while maximum aseptic cultures (66.67%) in shoot tip were recorded with 8% NaOCl for 20 minutes as well as 12% NaOCl for 10 minutes followed by 8% NaOCl for 30 minutes (63.33%). However, in both the explants, minimum aseptic cultures (0.00%) were recorded under control.

4.3.1 Effect of mercuric chloride ($HgCl_2$) on per cent aseptic cultures in apple clonal rootstocks during May 2018

The data pertaining to the effect of mercuric chloride ($HgCl_2$) on per cent aseptic cultures during the month of May 2018 is presented in table 4.3.1 and figure 4.5. The maximum aseptic cultures (73.33%) in clonal apple rootstock MM106 were recorded under 0.1% $HgCl_2$ for 4 minutes which was significantly superior over rest of the treatments and was followed by 0.1% $HgCl_2$ for 6 minutes (66.67%), 0.1% $HgCl_2$ for 2 minutes (56.67%) and 0.2% $HgCl_2$ for 2 minutes (51.67%), whereas the minimum aseptic cultures (0.00%) were recorded under control (without sterilizing agents). Between the two explants (shoot tip and nodal segment), nodal segment recorded (43.67%) aseptic cultures which were significantly superior to the shoot tip (35.67%). The interaction data revealed that nodal segment explants under 0.1% $HgCl_2$ for 4 minutes recorded highest aseptic cultures (76.67%) followed by 0.1% $HgCl_2$ for 6 minutes (70.00%), while in shoot tip maximum aseptic cultures (70.00%) were recorded under 0.1% $HgCl_2$ for 4 minutes followed by 0.1% $HgCl_2$ for 6 minutes (63.33%). However, minimum aseptic cultures (0.00%) were recorded under control for both explants.

The clonal apple rootstock MM111 on surface sterilization with mercuric chloride ($HgCl_2$) also showed a significant effect with respect to per cent aseptic cultures. Treatment of 0.1% $HgCl_2$ for 4 minutes gave maximum aseptic cultures (76.67%) which was significantly superior over rest of the treatments and was followed by 0.1% $HgCl_2$ for 6 minutes (70.00%), 0.1% $HgCl_2$ for 2 minutes (60.00%)

and 0.2% $HgCl_2$ for 2 minutes (55.00%), whereas the minimum aseptic cultures (0.00%) were recorded under control. Between the explants, nodal segment gave maximum average value (47.00%) for aseptic cultures which was significantly superior to shoot tips (39.00%). In the interaction effect, nodal segment explants under 0.1% $HgCl_2$ for 4 minutes gave maximum aseptic cultures (80.00%) followed by 73.33% with 0.1% $HgCl_2$ for 6 minutes, while maximum aseptic cultures in shoot tip (73.33%) were recorded under 0.1% $HgCl_2$ for 4 minutes followed by 0.1% $HgCl_2$ for 6 minutes (66.67%). However, minimum aseptic cultures in both explants (0.00%) were recorded under control.

4.3.2 Effect of sodium hypochlorite (NaOCl) on per cent aseptic cultures in apple clonal rootstocks during May 2018

The perusal of data presented in table 4.3.2 and illustrated in figure 4.6 revealed that the different treatments of sodium hypochlorite (NaOCl) showed a significant effect on the per cent aseptic cultures in clonal apple rootstock MM106 and MM111 during the month of May 2018. In clonal apple rootstock MM106, 8% NaOCl for 20 minutes gave maximum aseptic cultures (65.00%) which was significantly higher among all other treatments and was followed by 8% NaOCl for 30 minutes (60.00%) and 8% NaOCl for 10 minutes (56.67%), while minimum aseptic cultures (0.00%) were obtained under control (without sterilizing agents). Between thetwo explants, highest aseptic cultures (42.67%) were recorded in nodal segment which was significantly higher than the shoot tip (34.67%). The interaction data indicated that nodal segment explants gave maximum aseptic cultures (70.00%) with 8% NaOCl for 20 minutes followed by 8% NaOCl for 30 minutes (63.33%), whereas the shoot tip recorded 60.00 per cent aseptic cultures which was statistically at par with 8% NaOCl for 30 minutes (56.67%) followed by 8% NaOCl for 10 minutes (53.33%). However, minimum aseptic cultures (0.00%) were obtained under control.

The maximum aseptic cultures (66.67%) in clonal apple rootstock MM111 was recorded with 8% NaOCl for 20 minutes which was significantly superior to all other treatments and was followed by 60.00 per cent with 8% NaOCl for 30 minutes and 58.33 per cent with 8% NaOCl for 10 minutes, whereas the minimum aseptic cultures (0.00%) were recorded under control. Between the two explants, maximum aseptic cultures (44.33%) were recorded by nodal segment explants which were

Table 4.4.1: Effect of mercuric chloride ($HgCl_2$) on per cent survival cultures in apple clonal rootstocks during March 2018

Treatment	Duration (min.)	MM106			MM111		
		Shoot tip	Nodal segment	Mean	Shoot tip	Nodal segment	Mean
Control	-	0.00 (0.00)*	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
$HgCl_2$ (0.05%)	2	6.67 (14.96)	6.67 (14.96)	6.67 (14.96)	6.67 (14.96)	6.67 (14.96)	6.67 (14.96)
$HgCl_2$ (0.05%)	4	6.67 (14.96)	10.00 (18.00)	8.34 (16.48)	6.67 (14.96)	16.33 (23.76)	11.50 (19.36)
$HgCl_2$ (0.05%)	6	13.33 (21.32)	10.00 (18.00)	11.67 (19.66)	13.33 (21.32)	23.34 (28.83)	18.30 (25.07)
$HgCl_2$ (0.1%)	2	20.00 (26.49)	30.67 (33.61)	25.33 (30.05)	25.33 (30.19)	36.00 (36.84)	30.70 (33.51)
$HgCl_2$ (0.1%)	4	43.33 (41.14)	49.67 (44.79)	46.50 (42.97)	46.66 (43.06)	52.00 (46.13)	49.30 (44.59)
$HgCl_2$ (0.1%)	6	20.00 (26.50)	30.34 (33.38)	25.17 (29.94)	23.23 (28.83)	33.67 (35.44)	28.50 (32.13)
$HgCl_2$ (0.2%)	2	6.67 (14.96)	16.66 (24.02)	11.67 (19.49)	10.33 (18.74)	20.00 (26.50)	15.20 (22.62)
$HgCl_2$ (0.2%)	4	6.67 (14.96)	13.67 (21.61)	10.17 (18.28)	6.67 (14.96)	17.33 (24.57)	12.00 (19.76)
$HgCl_2$(0.2%)	6	6.67 (14.96)	6.67 (14.96)	6.67 (14.96)	6.67 (14.96)	13.63 (21.58)	10.20 (18.27)
Mean		13.00 (17.52)	17.44 (20.53)		14.57 (20.20)	21.90 (25.86)	
$CD_{(0.05)}$	Explant (E) = 1.05 Sterilant (S) = 2.35 E × S = 3.33				Explant (E) = 0.91 Sterilant (S) = 2.05 E × S = 2.90		

*Figures in parenthesis are arcsine transformed values

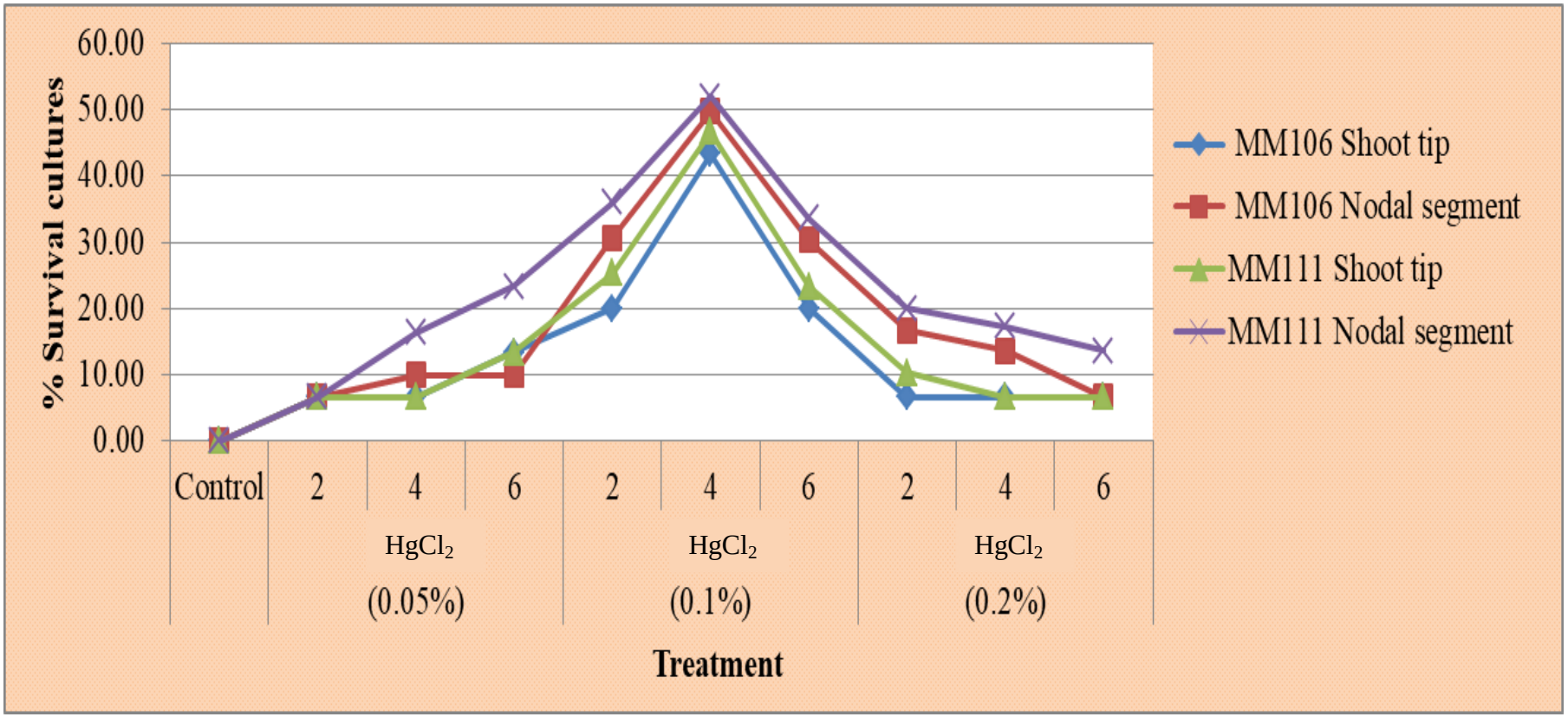


Figure 4.7: Effect of mercuric chloride ($HgCl_2$) on per cent survival cultures of clonal apple rootstocks during March 2018

Table 4.4.2: Effect of sodium hypochlorite (NaOCl) on per cent survival cultures in apple clonal rootstocks during March 2018

Treatment	Duration (min.)	MM106			MM111		
		Shoot tip	Nodal segment	Mean	Shoot tip	Nodal segment	Mean
Control	-	0.00 (0.00)*	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
NaOCl (3%)	**10**	6.67 (14.96)	13.00 (21.12)	9.84 (18.04)	10.67 (19.05)	16.66 (24.01)	13.67 (21.53)
NaOCl (3%)	**20**	10.00 (18.00)	10.00 (18.00)	10.00 (18.00)	13.67 (21.69)	13.33 (21.32)	13.50 (21.50)
NaOCl (3%)	**30**	6.67 (14.96)	16.33 (23.82)	11.50 (19.39)	10.67 (19.05)	19.66 (26.26)	15.17 (22.66)
NaOCl (8%)	**10**	20.67 (27.03)	30.33 (33.33)	25.50 (30.18)	23.33 (28.83)	33.00 (35.03)	28.17 (31.93)
NaOCl (8%)	**20**	30.33 (33.38)	33.00 (35.03)	31.67 (34.20)	40.00 (39.21)	47.00 (43.25)	43.50 (41.23)
NaOCl (8%)	**30**	23.33 (28.84)	33.67 (33.61)	28.50 (31.22)	26.43 (30.89)	36.66 (37.23)	31.55 (34.06)
NaOCl (12%)	**10**	6.67 (14.96)	16.66 (24.02)	11.67 (19.49)	10.33 (18.74)	20.00 (26.50)	15.17 (22.62)
NaOCl (12%)	**20**	6.67 (14.96)	6.67 (14.96)	6.67 (14.96)	6.67 (14.96)	6.67 (14.96)	6.67 (14.96)
NaOCl (12%)	**30**	6.67 (14.96)	6.67 (14.96)	6.67 (14.96)	6.67 (14.96)	6.67 (14.96)	6.67 (14.96)
Mean		11.77 (18.18)	16.33 (20.08)		14.84 (20.74)	19.97 (24.35)	
$CD_{(0.05)}$	Explant (E) = 0.97 Sterilant (S) = 2.17 E × S = 3.08				Explant (E) = 1.01 Sterilant (S) = 2.24 E × S = 3.17		

*Figures in parenthesis are arcsine transformed values

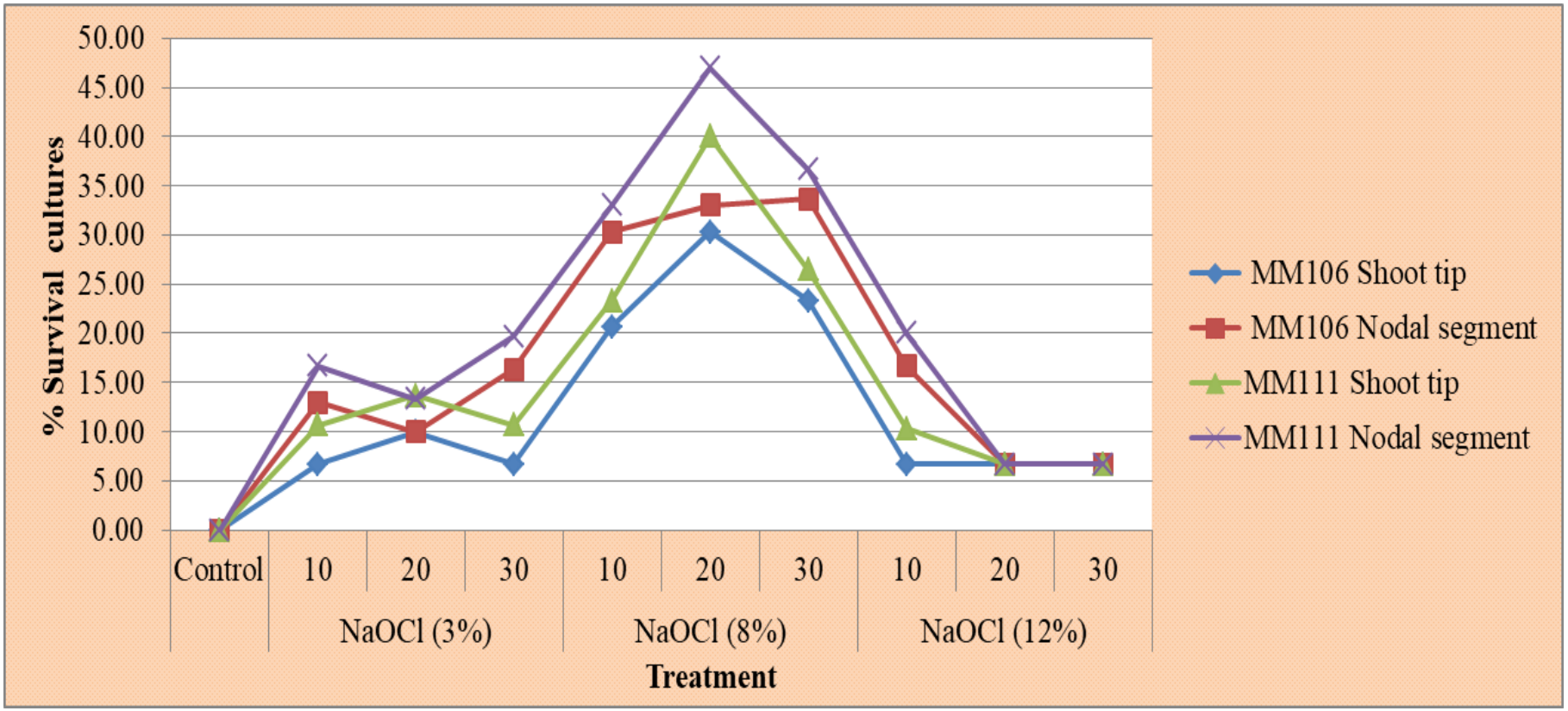


Figure 4.8: Effect of sodium chloride (NaOCl) on per cent survival cultures of clonal apple rootstocks during March 2018

Table 4.5.1: Effect of mercuric chloride ($HgCl_2$) on per cent survival cultures in apple clonal rootstocks during April 2018

Treatment	Duration (min.)	MM106			MM111		
		Shoot tip	Nodal segment	Mean	Shoot tip	Nodal segment	Mean
Control	-	0.00 (0.00)*	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
$HgCl_2$ (0.05%)	2	6.67 (14.96)	16.67 (24.01)	11.67 (19.48)	13.33 (21.32)	16.67 (24.01)	15.00 (22.67)
$HgCl_2$ (0.05%)	4	16.33 (23.82)	19.34 (26.07)	17.84 (24.94)	20.00 (26.55)	20.00 (26.50)	20.00 (26.52)
$HgCl_2$ (0.05%)	6	22.67 (28.41)	19.33 (26.07)	21.00 (27.24)	23.33 (28.87)	30.00 (33.17)	26.67 (31.02)
$HgCl_2$ (0.1%)	2	34.66 (36.05)	44.67 (41.92)	39.67 (38.98)	36.67 (37.23)	50.00 (44.98)	43.33 (41.11)
$HgCl_2$ (0.1%)	4	60.66 (51.14)	67.33 (55.12)	64.00 (53.13)	63.67 (52.92)	70.33 (56.97)	67.00 (54.94)
$HgCl_2$ (0.1%)	6	31.66 (34.22)	45.00 (42.11)	38.33 (38.16)	38.00 (38.03)	46.67 (43.06)	42.34 (40.55)
$HgCl_2$ (0.2%)	2	13.00 (21.12)	26.00 (30.60)	19.50 (25.86)	19.97 (26.46)	30.00 (33.17)	24.98 (29.81)
$HgCl_2$ (0.2%)	4	16.00 (23.39)	19.34 (26.07)	17.67 (24.73)	20.00 (26.55)	26.67 (31.05)	23.33 (28.80)
$HgCl_2$ (0.2%)	6	13.33 (21.32)	20.33 (26.73)	16.83 (24.02)	17.33 (24.51)	23.33 (28.83)	20.33 (26.67)
Mean		21.50 (25.44)	27.80 (29.86)		25.23 (28.24)	31.37 (32.17)	
$CD_{(0.05)}$	Explant (E) = 0.85 Sterilant (S) = 1.90 E × S = 2.69				Explant (E) = 0.97 Sterilant (S) = 2.17 E × S = 3..07		

*Figures in parenthesis are arcsine transformed values

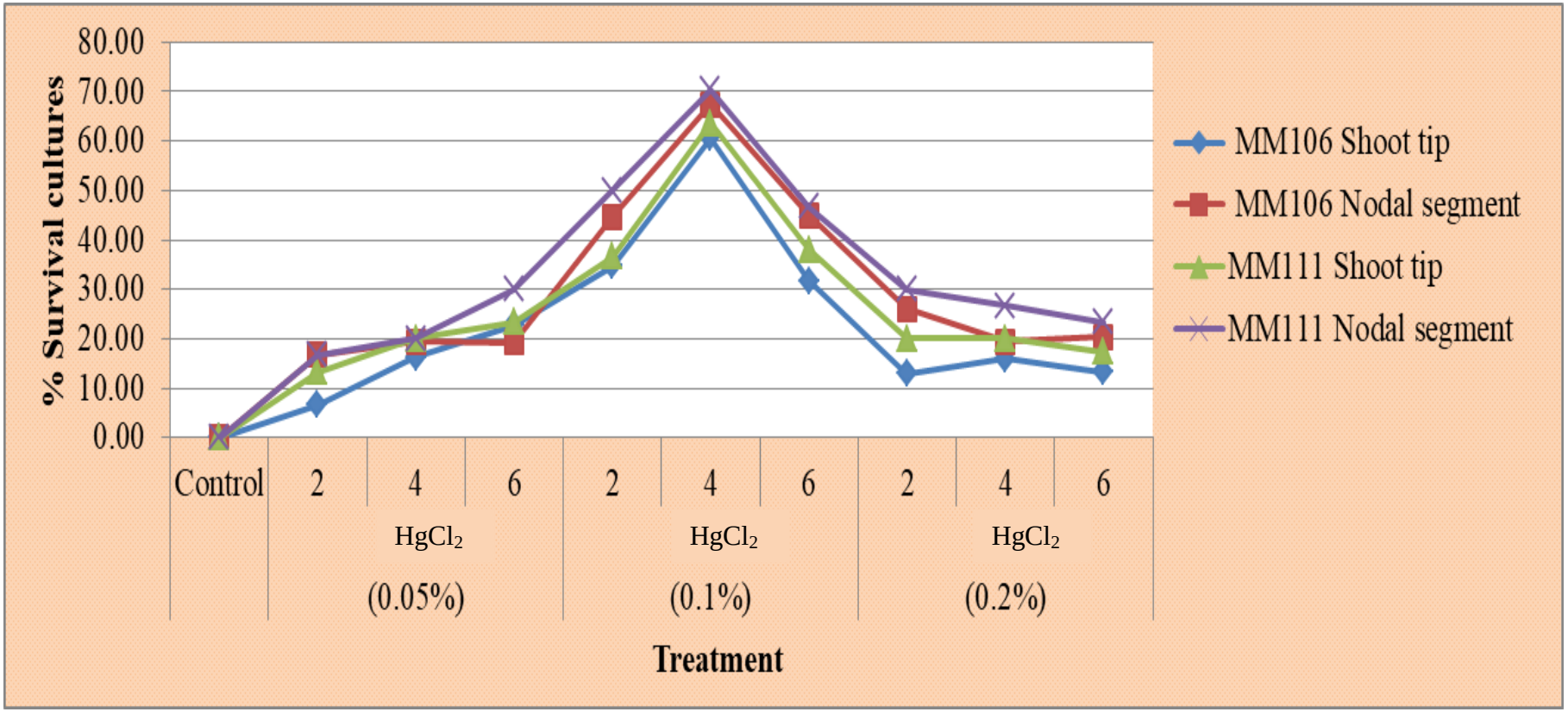


Figure 4.9: Effect of mercuric chloride ($HgCl_2$) on per cent survival cultures of clonal apple rootstocks during April 2018

Table 4.5.2: Effect of sodium hypochlorite (NaOCl) on per cent survival cultures in apple clonal rootstocks during April 2018

Treatment	Duration (min.)	MM106			MM111		
		Shoot tip	Nodal segment	Mean	Shoot Tip	Nodal segment	Mean
Control	-	0.00 (0.00)*	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
NaOCl (3%)	**10**	6.67 (14.96)	13.00 (21.12)	9.83 (18.04)	10.33 (18.56)	13.67 (21.61)	12.00 (20.08)
NaOCl (3%)	**20**	9.33 (17.76)	16.67 (24.01)	13.00 (20.92)	16.67 (24.01)	18.67 (25.57)	17.67 (24.79)
NaOCl (3%)	**30**	18.67 (25.57)	26.67 (31.05)	22.67 (28.31)	20.00 (26.50)	26.67 (31.05)	23.33 (28.77)
NaOCl (8%)	**10**	32.66 (34.84)	41.67 (40.18)	37.17 (37.51)	33.33 (35.23)	48.00 (43.83)	40.67 (39.53)
NaOCl (8%)	**20**	49.44 (44.66)	60.00 (50.75)	54.72 (47.71)	53.67 (47.08)	56.67 (48.81)	55.17 (47.95)
NaOCl (8%)	**30**	35.00 (36.22)	44.96 (42.09)	39.98 (39.15)	36.67 (37.25)	50.00 (44.98)	43.34 (41.11)
NaOCl (12%)	**10**	16.67 (24.01)	27.00 (31.27)	21.83 (27.64)	19.33 (25.97)	30.00 (33.17)	24.67 (29.57)
NaOCl (12%)	**20**	16.00 (23.39)	20.00 (26.55)	18.00 (24.97)	18.67 (25.57)	23.33 (28.87)	21.00 (27.22)
NaOCl (12%)	**30**	13.33 (21.40)	16.67 (24.01)	15.00 (22.71)	13.67 (21.61)	20.00 (26.50)	16.84 (24.05)
Mean		19.78 (24.28)	26.66 (29.13)		22.23 (26.18)	28.70 (30.44)	
$CD_{(0.05)}$	Explant (E) = 0.97 Sterilant(S) = 2.17 E × S = 3.07				Explant (E) = 1.26 Sterilant (S) = 2.82 E × S = 3.99		

*Figures in parenthesis are arcsine transformed values

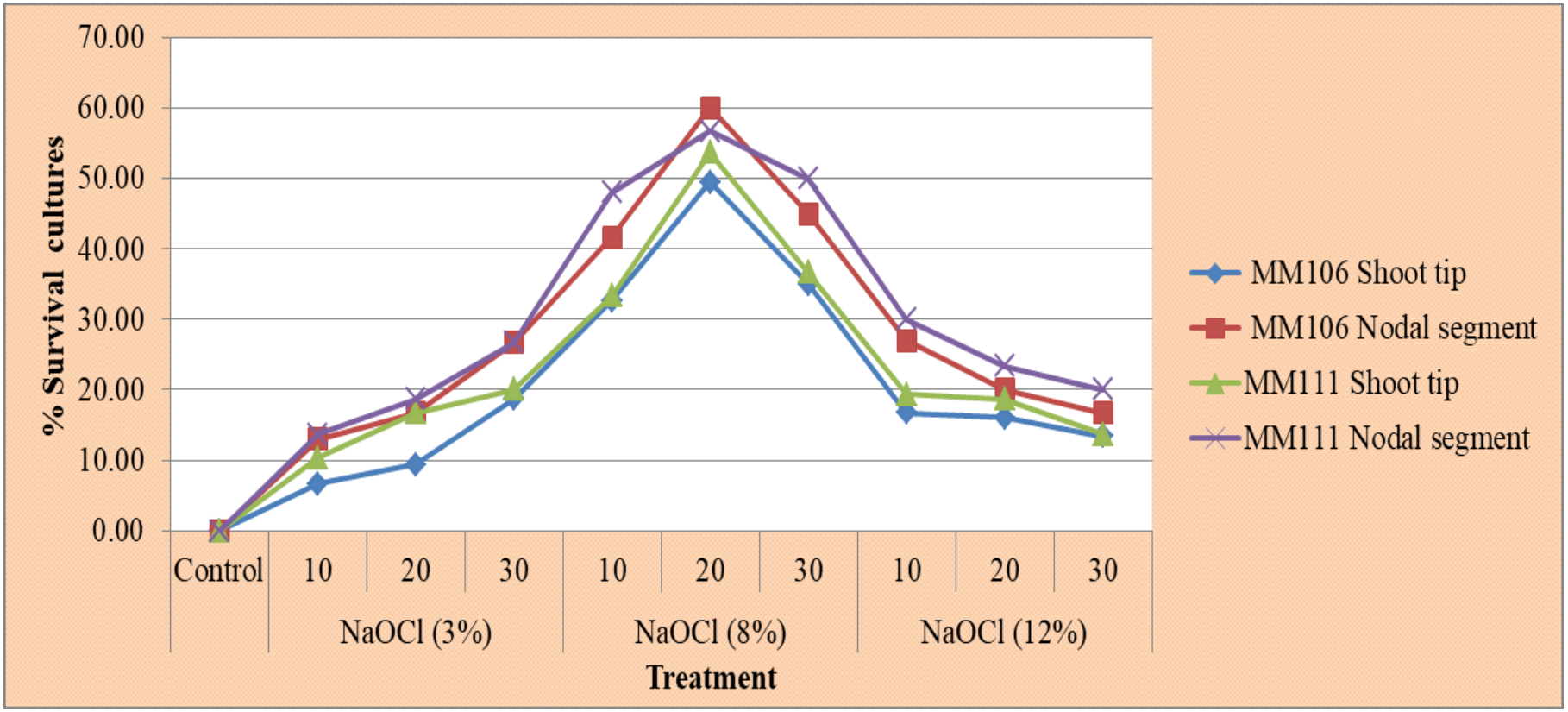


Figure 4.10: Effect of sodium chloride (NaOCl) on per cent survival cultures of clonal apple rootstocks during April 2018

Table 4.6.1: Effect of mercuric chloride ($HgCl_2$) on per cent survival cultures in apple clonal during May 2018

Treatment	Duration (min.)	MM106			MM111		
		Shoot tip	Nodal segment	Mean	Shoot tip	Nodal segment	Mean
Control	-	0.00 (0.00)*	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
$HgCl_2$ (0.05%)	2	6.67 (14.96)	10.77 (19.04)	8.72 (17.00)	6.67 (14.96)	13.33 (21.32)	10.00 (18.14)
$HgCl_2$ (0.05%)	4	10.33 (18.74)	13.33 (21.32)	11.83 (20.03)	10.00 (18.42)	17.00 (24.10)	13.50 (21.26)
$HgCl_2$(0.05%)	6	16.67 (24.01)	13.34 (21.40)	15.00 (22.56)	20.00 (26.50)	30.33 (33.38)	25.17 (29.94)
$HgCl_2$ (0.1%)	2	20.67 (27.01)	34.00 (35.57)	27.34 (22.70)	26.33 (30.84)	39.33 (38.71)	32.83 (34.77)
$HgCl_2$ (0.1%)	4	50.00 (44.98)	56.34 (48.62)	53.17 (46.80)	55.13 (47.92)	56.67 (48.81)	55.90 (48.37)
$HgCl_2$ (0.1%)	6	20.00 (26.50)	23.33 (28.83)	21.67 (27.66)	23.33 (28.87)	30.30 (33.40)	26.82 (31.13)
$HgCl_2$ (0.2%)	2	13.67 (21.61)	23.67 (29.07)	18.67 (25.34)	20.00 (26.50)	26.67 (31.05)	23.33 (28.78)
$HgCl_2$ (0.2%)	4	10.33 (18.56)	20.00 (26.49)	15.17 (22.52)	17.33 (24.58)	26.63 (31.02)	21.98 (27.80)
$HgCl_2$ (0.2%)	6	10.00 (18.00)	16.67 (24.01)	13.34 (21.01)	6.67 (14.96)	23.33 (28.87)	15.00 (21.91)
Mean		15.83 (21.43)	21.14 (25.43)		18.55 (23.35)	26.36 (29.07)	
$CD_{(0.05)}$	Explant (E) = 1.28 Sterilant (S) = 2.86 E × S = 4.05				Explant (E) = 1.17 Sterilant (S) = 2.62 E × S = 3.70		

*Figures in parenthesis are arcsine transformed values

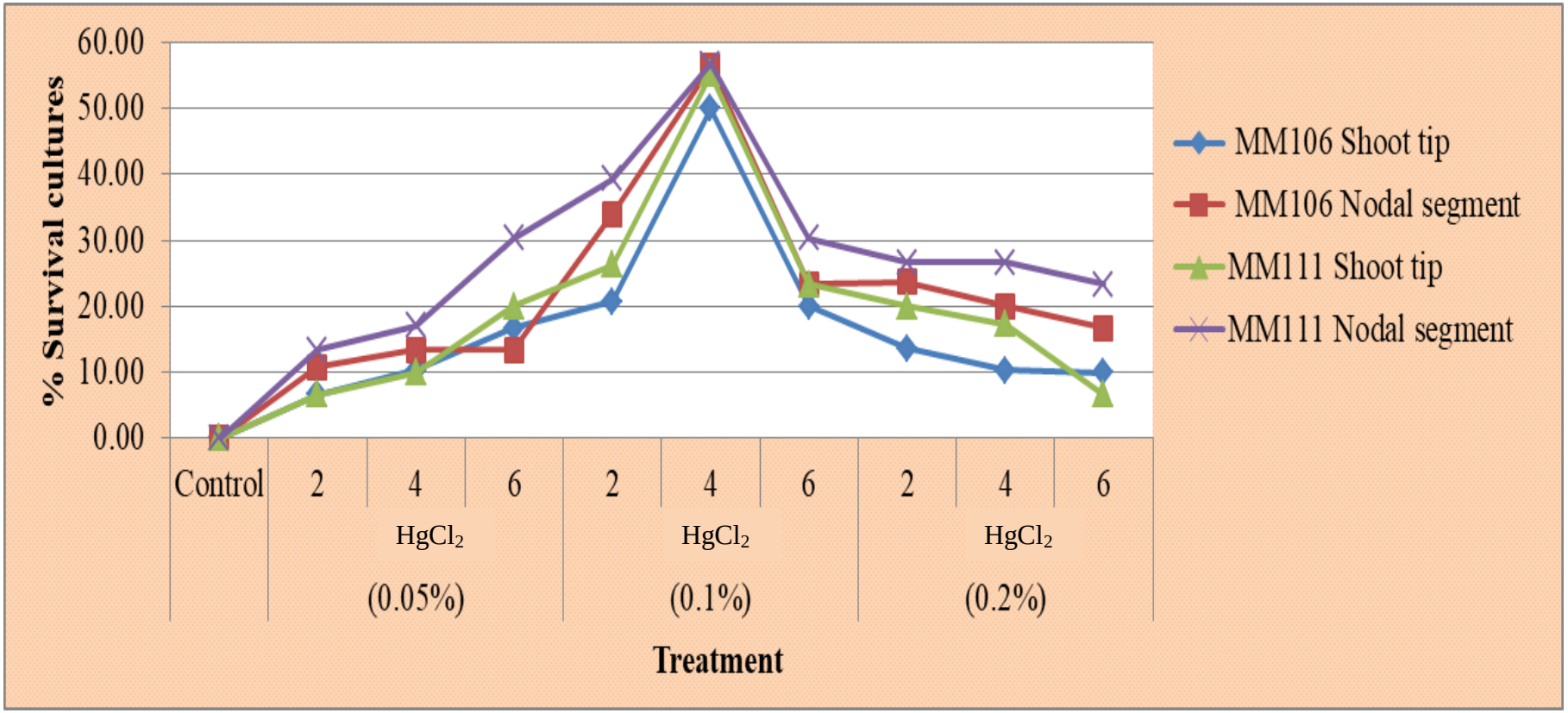


Figure 4.11: Effect of mercuric chloride ($HgCl_2$) on per cent survival cultures of clonal apple rootstocks during May 2018

Table 4.6.2: Effect of sodium hypochlorite (NaOCl) on per cent survival cultures in apple clonal rootstocks during May 2018

Treatment	Duration (min.)	MM106			MM111		
		Shoot tip	Nodal segment	Mean	Shoot tip	Nodal segment	Mean
Control	-	0.00 (0.00)*	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
NaOCl (3%)	**10**	6.67 (14.96)	6.67 (14.96)	6.67 (14.96)	6.67 (14.96)	10.00 (18.42)	8.34 (16.69)
NaOCl (3%)	**20**	6.67 (14.96)	6.67 (14.96)	6.61 (14.96)	13.33 (21.40)	16.67 (23.84)	15.00 (22.62)
NaOCl (3%)	**30**	13.33 (21.30)	13.00 (21.04)	13.17 (21.17)	16.67 (24.09)	26.67 (31.05)	21.67 (27.57)
NaOCl (8%)	**10**	22.89 (28.56)	33.00 (34.98)	27.94 (31.77)	33.33 (35.23)	46.67 (43.06)	40.00 (39.15)
NaOCl (8%)	**20**	39.67 (39.01)	46.66 (43.06)	43.17 (41.04)	50.00 (44.98)	53.33 (46.89)	51.67 (45.93)
NaOCl (8%)	**30**	26.00 (30.63)	36.33 (37.04)	31.17 (33.84)	34.67 (36.05)	46.67 (43.06)	40.67 (39.56)
NaOCl (12%)	**10**	10.00 (18.42)	20.00 (26.50)	15.00 (22.46)	20.00 (26.50)	26.67 (31.05)	23.33 (28.77)
NaOCl (12%)	**20**	10.33 (18.74)	19.33 (26.07)	14.83 (22.40)	16.67 (24.01)	18.67 (25.57)	17.67 (24.79)
NaOCl (12%)	**30**	10.33 (18.74)	16.33 (23.76)	13.33 (21.25)	10.00 (18.42)	23.33 (28.83)	16.67 (23.63)
Mean		14.59 (20.53)	19.80 (24.24)		20.13 (24.56)	26.25 (29.18)	
$CD_{(0.05)}$	Explant (E) = 0.82 Sterilant (S) = 1.84 E × S = 2.60				Explant (S) = 0.92 Sterilant (S) = 2.07 E × S = 2.93		

*Figures in parenthesis are arcsine transformed values

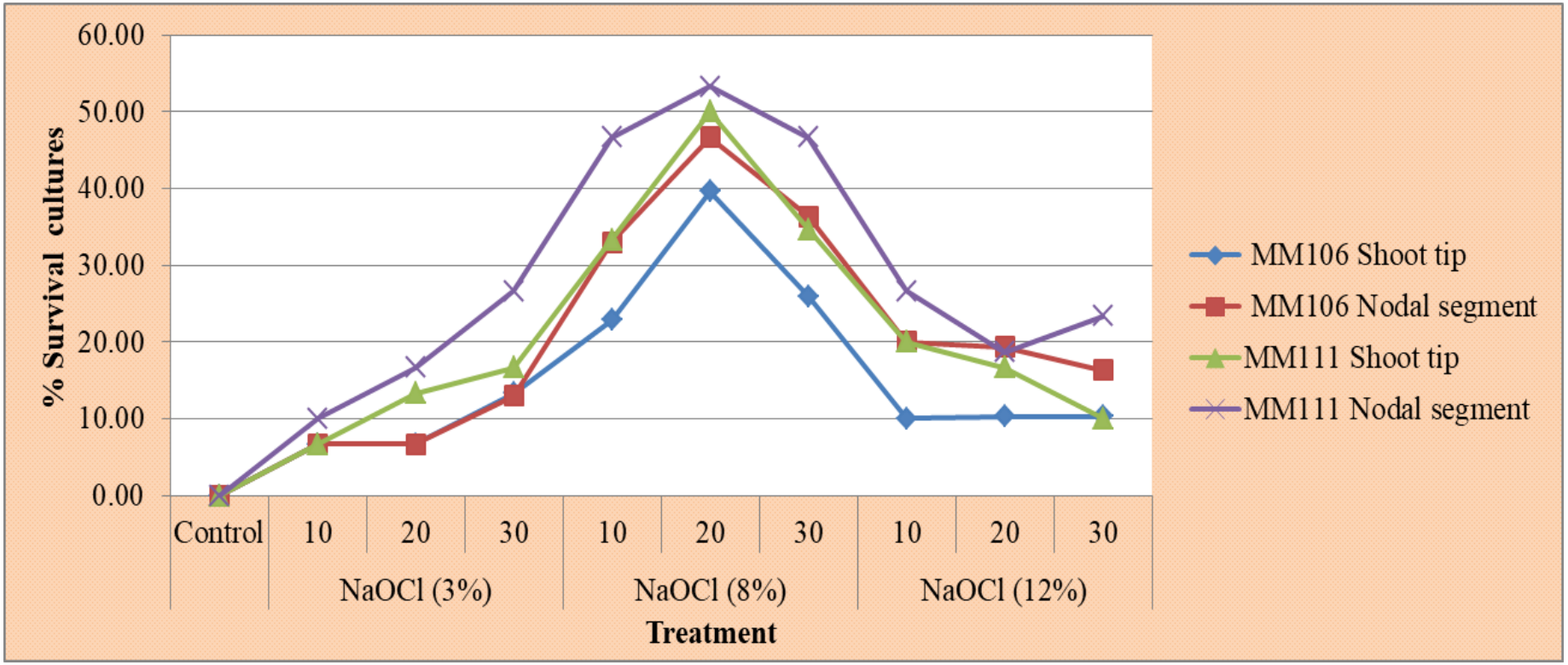


Figure 4.12: Effect of sodium chloride (NaOCl) on per cent survival cultures of clonal apple rootstocks during May 2018

significantly superior to the shoot tip (35.33%). The interaction data revealed that nodal segment explants with 8% NaOCl for 20 minutes gave maximum aseptic cultures (73.33%) followed by 8% NaOCl for 30 minutes (63.33%), while in shoot tip (60.00%) aseptic cultures was recorded with 8% NaOCl for 20 minutes followed by 56.67 per cent with 8% NaOCl for 10 minutes as well as 8% NaOCl for 30 minutes. However, in both the explants, minimum aseptic cultures (0.00%) were recorded under control.

4.4.1 Effect of mercuric chloride ($HgCl_2$) on per cent survival cultures in apple clonal rootstocks during March 2018

The data presented in table 4.4.1 and figure 4.7 showed that different treatments of mercuric chloride ($HgCl_2$) showed a significant effect on per cent survival of cultures during the month of March 2018. In clonal apple rootstock MM106, 0.1% $HgCl_2$ for 4 minutes recorded maximum survival of cultures (46.50%) which was significantly higher among all other treatments and was followed by 0.1% $HgCl_2$ for 2 minutes (25.33), 0.1% $HgCl_2$ for 6 minutes (25.17%) and 0.05% $HgCl_2$ for 6 minutes (11.67%). The minimum survival of cultures (0.00%) was recorded under control (without sterilizing agents). Between the explants (shoot tip and nodal segment), higher survival of cultures (17.44%) was recorded by nodal segment which was significantly higher than the shoot tip (13.00%). In the interaction effect, 0.1% $HgCl_2$ for 4 minutes gave maximum survival of cultures (49.67%) for nodal segment followed by 0.1% $HgCl_2$ for 2 minutes (30.67%), while shoot tip gave (43.33%) survival of cultures followed by 0.1% $HgCl_2$ for 2 minutes (20.00%). However, the lowest survival (0.00%) was recorded under control for both the explants.

In clonal apple rootstock MM111, 0.1% $HgCl_2$ for 4 minutes gave maximum survival of cultures (49.30%) which was significantly superior over rest of the treatments and was followed by 0.1% $HgCl_2$ for 2 minutes (30.70%), 0.1% $HgCl_2$ for 6 minutes (28.50%) and 0.05% $HgCl_2$ for 6 minutes (19.30%), whereas the minimum survival of cultures (0.00%) were recorded under control. Between the explants, nodal segment recorded maximum survival of cultures (21.90%) which was also significantly superior to the shoot tip (14.57%). In the interaction effect, nodal segment explants treated with 0.1% $HgCl_2$ for 4 minutes recorded maximum survival of cultures (52.00%) followed by 0.1% $HgCl_2$ for 2 minutes (36.00%), while shoot tip recorded 46.66% with 0.1% $HgCl_2$ for 4 minutes followed by 25.33% with 0.1%

$HgCl_2$ for 2 minutes. However, minimum survival of cultures (0.00%) was recorded under control for both explants.

4.4.2 Effect of sodium hypochlorite (NaOCl) on per cent survival cultures in apple clonal rootstocks during March 2018

The perusal of data presented in table 4.4.2 and figure 4.8 revealed that sodium hypochlorite (NaOCl) showed a significant influence on the per cent survival of cultures in clonal apple rootstocks MM106 and MM111 during the month of March 2018. In apple clonal rootstock MM106, 8% NaOCl for 20 minutes gave highest survival of cultures (31.67%) which was significantly higher among all other treatments and was followed by 8% NaOCl for 30 minutes (28.50%) and 8% NaOCl for 10 minutes (25.50%). The lowest survival of cultures (0.00%) was recorded under control (without sterilizing agents). Between the explants, nodal segment gave higher survival of cultures (16.33%) which was significantly higher than shoot tip (11.77%). In the interaction effect, nodal segment explants gave higher survival of cultures (33.67%) with 8% NaOCl for 30 minutes followed by 8% NaOCl for 20 minutes (33.00%), while higher survival of cultures (30.33%) in shoot tip was recorded with 8% NaOCl of 20 minutes followed by 8% NaOCl for 30 minutes (23.33%). However, the lowest survival of cultures (0.00%) for both explants was recorded under control.

In clonal apple rootstock MM111, 8% NaOCl for 20 minutes gave maximum survival of cultures (43.50%) which was found to be significantly superior over rest of the treatments and was followed by 8% NaOCl for 30 minutes (31.55%) and 8% NaOCl for 10 minutes (28.17%). However, minimum survival of cultures (0.00%) was recorded under control. Between the explants, nodal segment recorded maximum survival of cultures (19.97%) which was significantly superior to the shoot tip (14.84%). The interaction data revealed that nodal segment explants treated with 8% NaOCl for 20 minutes recorded maximum survival of cultures (47.00%) followed by 8% NaOCl for 30 minutes (36.66%), while maximum survival of cultures in shoot tip (40.00%) was recorded with 8% NaOCl for 20 minutes followed by 8% NaOCl for 30 minutes (26.43%). The minimum survival of cultures (0.00%) for both the explants was recorded under control.

4.5.1 Effect of mercuric chloride ($HgCl_2$) on per cent survival cultures in apple clonal rootstocks during April 2018

The data with respect to the effect of mercuric chloride ($HgCl_2$) on per cent survival of cultures during the month of April 2018 is presented in table 4.5.1 and figure 4.9 which showed a significant effect on per cent survival of cultures of clonal apple rootstocks MM106 and MM111. Treatment of 0.1% $HgCl_2$ for 4 minutes gave maximum survival of cultures (64.00%) in clonal apple rootstock MM106 which was significantly higher among all other treatments and was followed by 0.1% $HgCl_2$ for 2 minutes (39.67%), 0.1% $HgCl_2$ for 6 minutes (38.33%) and 0.05% $HgCl_2$ for 6 minutes (21.00%). The minimum survival of cultures (0.00%) was observed under control (without sterilizing agents). Between the explants (shoot tip and nodal segment), highest survival of cultures (27.80%) was recorded by nodal segment which was significantly higher than the shoot tip (21.50%). The interaction data indicated that treatment of nodal segment with 0.1% $HgCl_2$ for 4 minutes gave maximum survival of cultures (67.33%) followed by 0.1% $HgCl_2$ for 6 minutes (45.00%), while shoot tip gave (60.66%) survival of cultures followed by 0.1% $HgCl_2$ for 2 minutes (34.66%). However, the lowest survival of cultures (0.00%) was recorded under control for both explants.

In clonal apple rootstock MM111, maximum survival of cultures (67.00%) was obtained with 0.1% $HgCl_2$ for 4 minutes which was significantly superior over rest of the treatments and was followed by 0.1% $HgCl_2$ for 2 minutes (43.33%), 0.1% $HgCl_2$ for 6 minutes (42.34%) and 0.05% $HgCl_2$ for 6 minutes (26.67%). The minimum survival of cultures (0.00%) was recorded under control. Between the explants, nodal segment gave maximum survival of cultures (31.37%) with 0.1% $HgCl_2$ for 4 minutes which was significantly higher than the shoot tip (25.23%). The interaction study revealed nodal segment explants treated with 0.1% $HgCl_2$ for 4 minutes gave maximum survival of cultures (70.33%) followed by 0.1% $HgCl_2$ for 2 minutes (50.00%), while shoot tip recorded (63.67%) survival of cultures with 0.1% $HgCl_2$ for 4 minutes followed by 0.1% $HgCl_2$ for 2 minutes (38.00%). However, both the explants (shoot tip and nodal segment) recorded minimum survival of cultures (0.00%) under control.

4.5.2 Effect of sodium hypochlorite (NaOCl) on per cent survival cultures in apple clonal rootstocks during April 2018

The data enumerated in table 4.10 and figure 4.10 revealed that different treatments of sodium hypochlorite (NaOCl) showed a significant influence on per cent survival of cultures of clonal apple rootstocks MM106 and MM111 during the month of April 2018. In apple clonal rootstock MM106, 8% NaOCl for 20 minutes gave highest survival of cultures (54.72%) which was significantly higher among all other treatments and was followed by 8% NaOCl for 30 minutes (39.98%) and 8% NaOCl for 10 minutes (37.17%). The lowest survival of cultures (0.00%) was recorded under control (without sterilizing agents). Between the two explants, nodal segment gave highest survival of cultures (26.66%) which was significantly higher than the shoot tip (19.78%). In the interaction effect, nodal segment explants gave highest survival of cultures (60.00%) with 8% NaOCl for 20 minutes followed by 44.96 per cent with 8% NaOCl for 30 minutes, while higher survival of cultures (49.44%) in shoot tip were recorded with 8% NaOCl for 20 minutes followed by 35.00 per cent with 8% NaOCl for 30 minutes. However, lowest survival of cultures (0.00%) for both the explants was recorded under control.

In MM111 rootstock, 8% NaOCl for 20 minutes recorded maximum survival of cultures (55.17%) which was significantly higher among all other treatments and was followed by 8% NaOCl for 30 minutes (43.34%) and 8% NaOCl for 10 minutes (40.67%), whereas the minimum survival of cultures (0.00%) was recorded under control. Between the explants, nodal explants recorded maximum survival of cultures (28.70%) which was significantly higher than shoot tip (22.23%). The interaction data revealed that maximum survival of cultures (56.67%) in nodal segment were recorded with 8% NaOCl for 20 minutes followed by 8% NaOCl for 30 minutes (50.00%), while shoot tip recorded (53.67%) with 8% NaOCl for 20 minutes followed by 8% NaOCl for 30 minutes (36.67%). However, in both the explants, minimum survival of cultures (0.00%) was recorded under control.

4.6.1 Effect of mercuric chloride ($HgCl_2$) on per cent survival cultures in apple clonal rootstocks during May 2018

The data enumerated in table 4.6.1 and figure 4.11 revealed that different treatments of mercuric chloride ($HgCl_2$) showed a significant influence on per cent

survival of culture of MM106 and MM111 rootstocks during May 2018. Treatment of 0.1% $HgCl_2$ for 4 minutes gave maximum survival of cultures (53.17%) in clonal apple rootstock MM106 which was significantly superior over rest of the treatments and was followed by 0.1% $HgCl_2$ for 2 minutes (27.34%), 0.1% $HgCl_2$ for 6 minutes (21.67%) and 0.2% $HgCl_2$ for 2 minutes (18.67%). The lowest survival of cultures (0.00%) was recorded under control (without sterilizing agents). Between the two explants (shoot tip and nodal segment), highest survival of cultures (21.14%) was recorded by nodal segment which was significantly higher than the shoot tip (15.83%). In the interaction effect, 0.1% $HgCl_2$ for 4 minutes gave maximum survival of cultures (56.34%) in nodal segment followed by 0.1% $HgCl_2$ for 2 minutes (34.00%), while shoot tip gave (50.00%) survival of cultures followed by 0.1% $HgCl_2$ for 2 minutes (20.67%). However, the lowest survival of cultures (0.00%) was recorded under control for both explants.

In clonal apple rootstock MM111, maximum survival of cultures (55.90%) was obtained with 0.1% $HgCl_2$ for 4 minutes which was significantly higher than all other treatments and was followed by 0.1% $HgCl_2$ for 2 minutes (32.83%), 0.1% $HgCl_2$ for 6 minutes (26.82%) and 0.05% $HgCl_2$ for 6 minutes (25.17%). However, minimum survival of cultures (0.00%) was recorded under control. Between the explants, nodal segment recorded maximum survival of cultures (26.36%) which were significantly higher than the shoot tip (18.55%). In the interaction effect, nodal segment explants under 0.1% $HgCl_2$ for 4 minutes gave maximum survival of cultures (56.67%) followed by 0.1% $HgCl_2$ for 2 minutes (39.33%), while shoot tip recorded (55.13%) survival of cultures followed by 0.1% $HgCl_2$ for 2 minutes (26.33%). For both the explants, minimum survival of cultures (0.00%) was recorded under control.

4.6.2 Effect of sodium hypochlorite (NaOCl) on per cent survival cultures in apple clonal rootstocks during May 2018

The data presented in table 4.6.2 and illustrated in figure 4.12 revealed that sodium hypochlorite (NaOCl) showed a significant influence on per cent survival of cultures of clonal apple rootstocks i.e. MM106 and MM111 during May 2018. In clonal apple rootstock MM106, 8% NaOCl for 20 minutes gave higher value for survival of cultures (43.17%) which was significantly superior over rest of the treatments and was followed by 8% NaOCl for 30 minutes (31.17%) and 8% NaOCl

for 10 minutes (27.94%). The lowest survival of cultures (0.00%) was recorded under control (without sterilizing agents). Between the explants, nodal segment gave higher survival of cultures (19.80%) which was significantly higher than the shoot tip (14.59%). In the interaction effect, nodal segment explants gave highest survival of cultures (46.66%) with 8% NaOCl for 20 minutes followed by 36.33 per cent with 8% NaOCl for 30 minutes, while higher survival of cultures (39.67%) in shoot tip were recorded with 8% NaOCl for 20 minutes followed by 26.00 per cent with 8% NaOCl for 30 minutes. However, lowest survival of cultures (0.00%) for both the explants was recorded under control.

In MM106 rootstock, 8% NaOCl for 20 minutes recorded maximum survival of cultures (51.67%) which was significantly higher over rest of the treatments and was followed by 8% NaOCl for 30 minutes (40.67%) and 8% NaOCl for 10 minutes (40.00%), whereas the minimum survival of cultures (0.00%) was recorded under control. Between the two explants, nodal explants gave maximum survival of cultures (26.25%) which was significantly higher than the shoot tip (20.13%). The interaction data indicated that nodal segment explants treated with 8% NaOCl for 20 minutes gave highest survival of cultures i.e. 53.33 per cent followed by 8% NaOCl for 30 minutes (46.67%), while shoot tip recorded (50.00%) with 8% NaOCl for 20 minutes followed by 8% NaOCl for 30 minutes (34.67%). However, in both the explants, lowest survival of cultures (0.00%) was recorded under control.

4.7 Effect of antioxidants on per cent culture establishment of clonal apple rootstocks

The data presented in table 4.7 and illustrated in figure 4.13 revealed that different antioxidants showed a significant effect on per cent culture establishment of clonal apple rootstocks MM106 and MM111. The clonal apple rootstock MM106 recorded maximum culture establishment (30.00%) with media composition AM4 (MS + 10 g PVP) which was statistically at par with AM3 (MS + 5 g PVP) i.e. 28.88 per cent followed by 24.44 per cent in AM7 (MS + 10 g citric acid) and 23.33 per cent in AM6 (MS + 5 g citric acid). The MS basal medium showed minimum culture establishment (0.00%). Between the two different explants (shoot tip and nodal segment), nodal segment showed maximum culture establishment (24.11%) which was significantly higher than the shoot tip (17.11%). In the interaction effect, media composition AM4 showed maximum culture establishment (32.22%) in nodal

Table 4.7: Effect of antioxidants on per cent culture establishment of clonal apple rootstocks

Media Code	Treatment	MM106			MM111		
		Shoot tip	Nodal segment	Mean	Shoot tip	Nodal segment	Mean
AM1	Control: MS (Basal)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)*	0.00 (0.00)	0.00 (0.00)
AM2	MS + PVP (2.5 g)	16.66 (24.01)	25.55 (30.34)	21.11 (27.17)	27.78 (31.78)	36.66 (37.24)	32.22 (34.51)
AM3	MS + PVP (5 g)	26.66 (31.07)	31.11 (33.85)	28.88 (32.46)	34.55 (35.98)	46.66 (43.06)	40.61 (39.52)
AM4	MS + PVP (10 g)	27.77 (31.78)	32.22 (34.54)	30.00 (33.16)	35.33 (36.45)	47.88 (43.76)	41.61 (40.11)
AM5	MS + Citric acid (2.5 g)	11.11 (19.42)	18.89 (25.73)	15.00 (22.57)	21.55 (27.64)	31.33 (34.01)	26.44 (30.82)
AM6	MS + Citric acid (5 g)	18.88 (25.67)	27.77 (31.78)	23.33 (28.72)	28.89 (32.49)	37.78 (37.90)	33.34 (35.20)
AM7	MS + Citric acid (10 g)	20.00 (26.50)	28.88 (32.46)	24.44 (29.48)	30.00 (33.19)	38.89 (38.56)	34.45 (35.88)
AM8	MS + Ascorbic acid (2.5 g)	15.55 (23.18)	24.44 (29.60)	20.00 (26.39)	27.77 (31.78)	34.44 (35.91)	31.11 (33.84)
AM9	MS + Ascorbic acid (5 g)	16.66 (24.08)	25.56 (30.34)	21.11 (27.12)	28.89 (32.49)	35.55 (36.58)	32.22 (34.53)
AM10	MS + Ascorbic acid (10 g)	17.77 (24.90)	26.67 (31.08)	22.22 (27.99)	30.00 (33.17)	36.66 (37.24)	33.33 (35.21)
	Mean	17.11 (23.06)	24.11 (27.97)		26.48 (29.49)	34.59 (34.43)	
	$CD_{(0.05)}$	Explant (E) = 0.86 Antioxidant (A) = 1.93 E × A = 2.72			Explant (E) = 0.54 Antioxidant (A) = 1.22 E × A = 1.72		

*PVP = Polyvinyl pyrrolidone

*Figures presented in parenthesis are arcsine transformed values

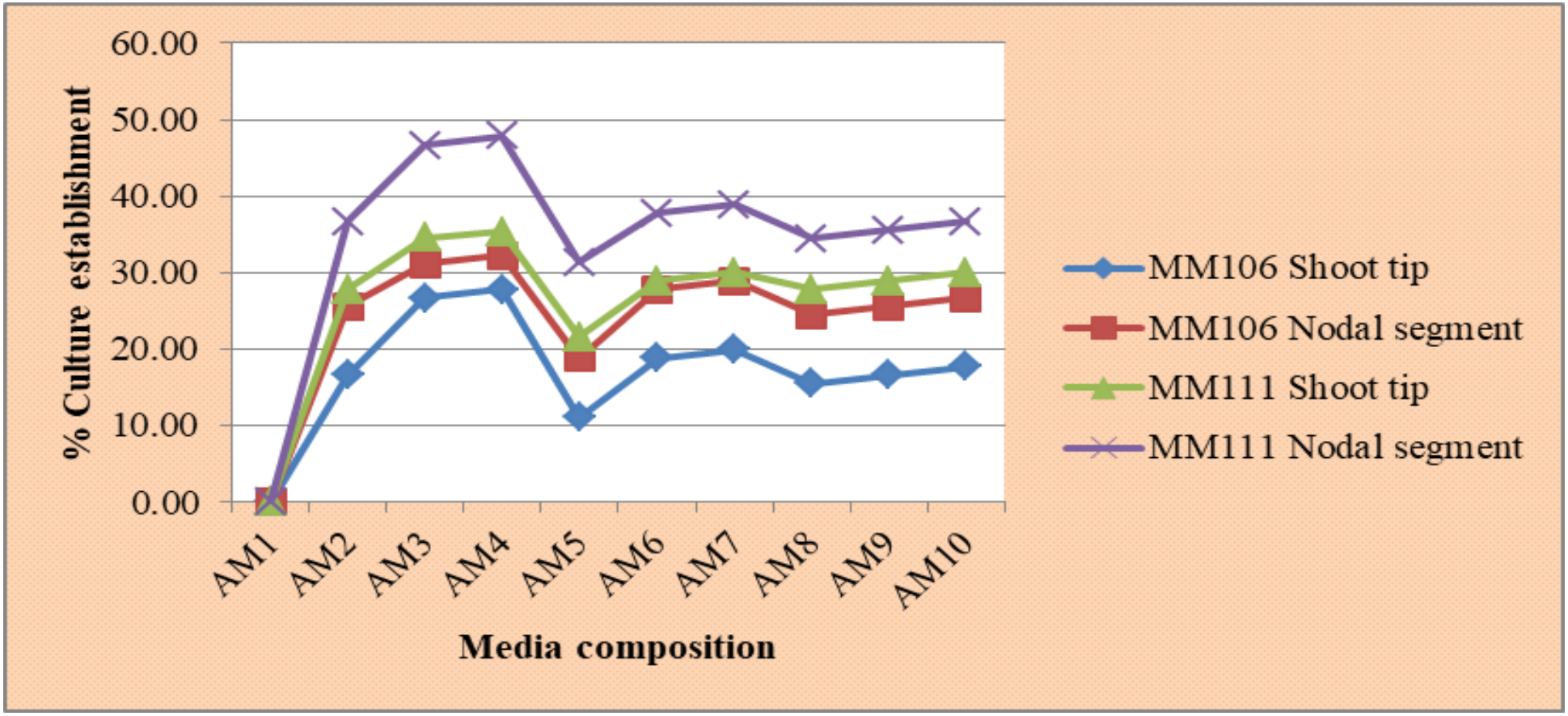


Figure 4.13: Effect of antioxidants on per cent culture establishment of clonal apple rootstocks

Table 4.8: Effect of mediacomposition on per cent culture establishment of clonal apple rootstocks

Media Code	Treatment	MM106			MM111		
		Shoot tip	Nodal segment	Mean	Shoot tip	Nodal segment	Mean
EM1	Control: MS (Basal)	0.00 (0.00)*	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
EM2	MS + BAP (1.0 mg/l)	46.66 (43.06)	56.66 (48.81)	51.66 (45.94)	60.00 (50.75)	75.56 (60.35)	67.78 (55.55)
EM3	MS + BAP (2.0 mg/l)	33.33 (35.24)	43.33 (41.15)	38.33 (38.19)	50.00 (44.98)	63.33 (52.72)	56.66 (48.85)
EM4	MS + BAP (3.0 mg/l)	23.33 (28.83)	26.66 (31.04)	25.00 (29.94)	36.66 (37.24)	46.66 (43.06)	41.66 (40.15)
EM5	MS + GA_3 (1.0 mg/l)	30.00 (33.19)	35.55 (36.58)	32.78 (34.88)	40.00 (39.20)	54.44 (47.53)	47.22 (43.36)
EM6	MS + GA_3 (2.0 mg/l)	26.66 (31.04)	23.33 (28.83)	25.00 (29.94)	30.00 (33.17)	43.33 (41.14)	36.66 (37.15)
EM7	MS + GA_3 (3.0 mg/l)	20.00 (26.50)	13.33 (21.30)	16.66 (23.90)	20.00 (26.50)	30.00 (33.17)	25.00 (29.83)
EM8	MS + BAP (1.0 mg/l) + GA_3 (1.0 mg/l)	47.77 (43.70)	57.77 (49.45)	52.77 (46.57)	61.11 (51.40)	76.66 (61.12)	68.89 (56.26)
EM9	MS + BAP (1.0 mg/l) + GA_3 (2.0 mg/l)	23.33 (28.83)	30.00 (33.17)	26.66 (31.01)	26.66 (31.04)	40.00 (39.21)	33.33 (35.13)
EM10	MS + BAP (1.0 mg/l) + GA_3 (3.0 mg/l)	16.66 (24.01)	20.00 (26.50)	18.33 (25.25)	16.66 (24.08)	27.77 (31.78)	22.22 (27.93)
EM11	MS + BAP (2.0 mg/l) + GA_3 (1.0 mg/l)	34.44 (35.91)	44.44 (41.78)	39.44 (38.85)	51.11 (45.61)	64.44 (53.37)	57.78 (49.49)
EM12	MS + BAP (2.0 mg/l) + GA_3 (2.0 mg/l)	20.00 (26.50)	20.00 (26.50)	20.00 (26.50)	23.33 (28.87)	33.33 (35.24)	28.33 (32.05)
EM13	MS + BAP (2.0 mg/l) + GA_3 (3.0 mg/l)	13.33 (21.30)	16.66 (24.08)	15.00 (22.69)	13.33 (21.40)	23.33 (28.87)	18.33 (25.13)
EM14	MS + BAP (3.0 mg/l) + GA_3 (1.0 mg/l)	31.11 (33.88)	36.66 (37.23)	33.89 (35.55)	41.11 (39.86)	55.55 (48.16)	48.33 (44.01)
EM15	MS + BAP (3.0 mg/l) + GA_3 (2.0 mg/l)	10.00 (18.42)	10.00 (18.42)	10.00 (18.42)	13.33 (21.40)	20.00 (26.50)	16.66 (23.95)
EM16	MS + BAP (3.0 mg/l) + GA_3 (3.0 mg/l)	6.66 (14.95)	6.66 (14.95)	6.66 (14.95)	10.00 (18.42)	16.66 (24.01)	13.33 (21.22)
	Mean	23.96 (27.83)	27.57 (29.98)		30.83 (32.12)	41.94 (39.14)	
	$CD_{(0.05)}$	Explant (E) = 0.83 Media (M) = 2.33 E × M = 3.29			Explant (E) = 0.64 Media (M) = 1.79 E × M = 2.54		

*Figures in parenthesis are arcsine transformed values

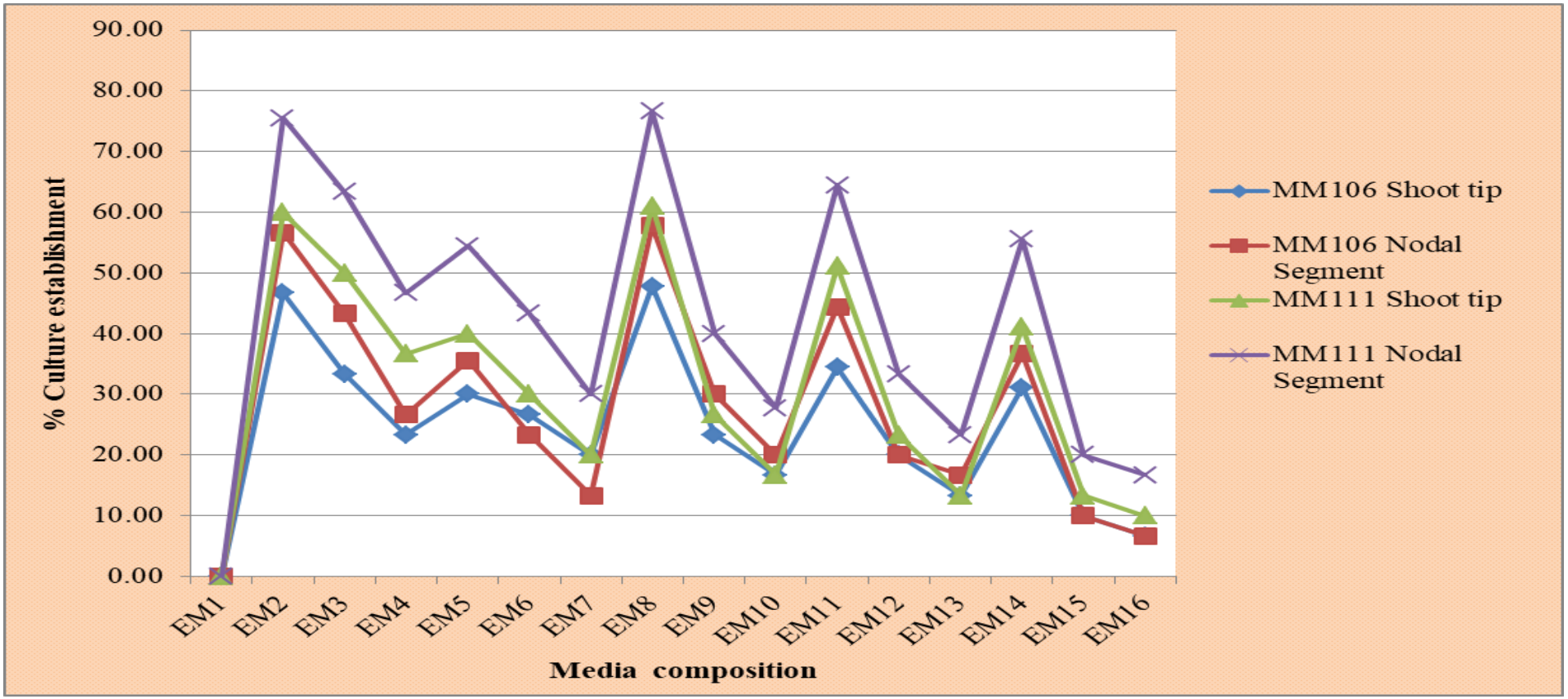


Figure 4.14: Effect of media composition on per cent culture establishment of clonal apple rootstocks

Table 4.9: Effect of media composition on number of days taken for culture establishment of clonal apple rootstocks

Media Code	Treatment	MM106			MM111		
		Shoot tip	Nodal segment	Mean	Shoot tip	Nodal segment	Mean
EM1	Control: MS (Basal)	-	-	-	-	-	-
EM2	MS + BAP (1.0 mg/l)	33.27	26.47	29.87	32.27	25.47	28.87
EM3	MS + BAP (2.0 mg/l)	33.80	27.73	30.77	33.33	27.53	30.43
EM4	MS + BAP (3.0 mg/l)	37.60	31.53	34.57	36.60	30.53	33.57
EM5	MS + GA_3 (1.0 mg/l)	34.27	28.13	31.20	33.43	27.67	30.55
EM6	MS + GA_3 (2.0 mg/l)	35.47	30.40	32.93	35.20	30.20	32.70
EM7	MS + GA_3 (3.0 mg/l)	38.53	32.47	35.50	38.27	32.27	35.27
EM8	MS + BAP (1.0 mg/l) + GA_3 (1.0 mg/l)	32.30	26.33	29.32	32.20	25.40	28.80
EM9	MS + BAP (1.0 mg/l) + GA_3 (2.0 mg/l)	35.63	30.63	33.13	35.43	30.40	32.92
EM10	MS + BAP (1.0 mg/l) + GA_3 (3.0 mg/l)	38.83	32.73	35.78	38.47	32.47	35.47
EM11	MS + BAP (2.0 mg/l) + GA_3 (1.0 mg/l)	33.53	27.50	30.52	33.27	27.43	30.35
EM12	MS + BAP (2.0 mg/l) + GA_3 (2.0 mg/l)	38.87	32.87	35.87	38.57	32.53	35.55
EM13	MS + BAP (2.0 mg/l) + GA_3 (3.0 mg/l)	39.20	33.23	36.22	38.93	32.83	35.88
EM14	MS + BAP (3.0 mg/l) + GA_3 (1.0 mg/l)	34.17	28.00	31.09	33.17	27.53	30.35
EM15	MS + BAP (3.0 mg/l) + GA_3 (2.0 mg/l)	40.17	34.37	37.27	39.33	33.33	36.33
EM16	MS + BAP (3.0 mg/l) + GA_3 (3.0 mg/l)	41.30	35.33	38.32	40.27	34.13	37.20
	Mean	36.46	30.52		35.92	29.98	
	$CD_{(0.05)}$	Explant (E) = 0.03 Media (M) = 0.10 E × M = 0.14			Explant (E) = 0.04 Media (M) = 0.11 E × M = 0.16		

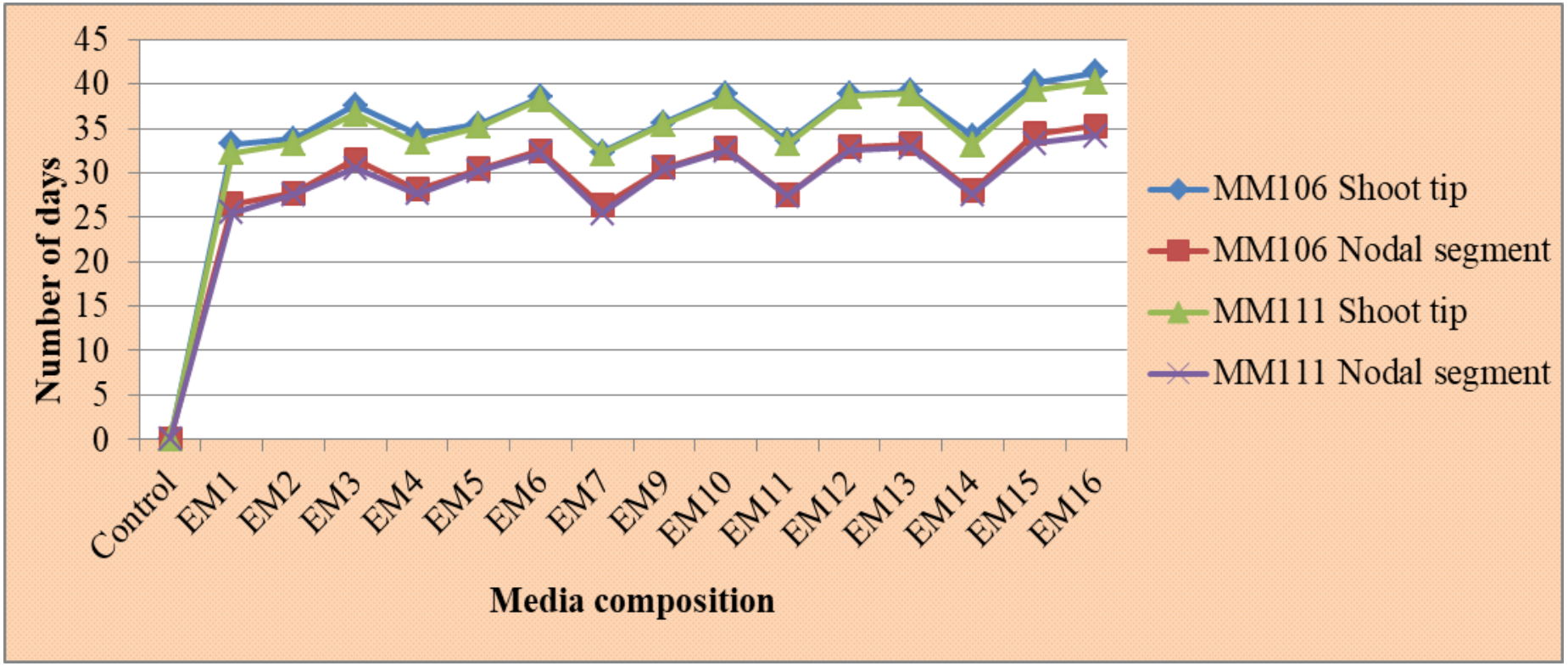
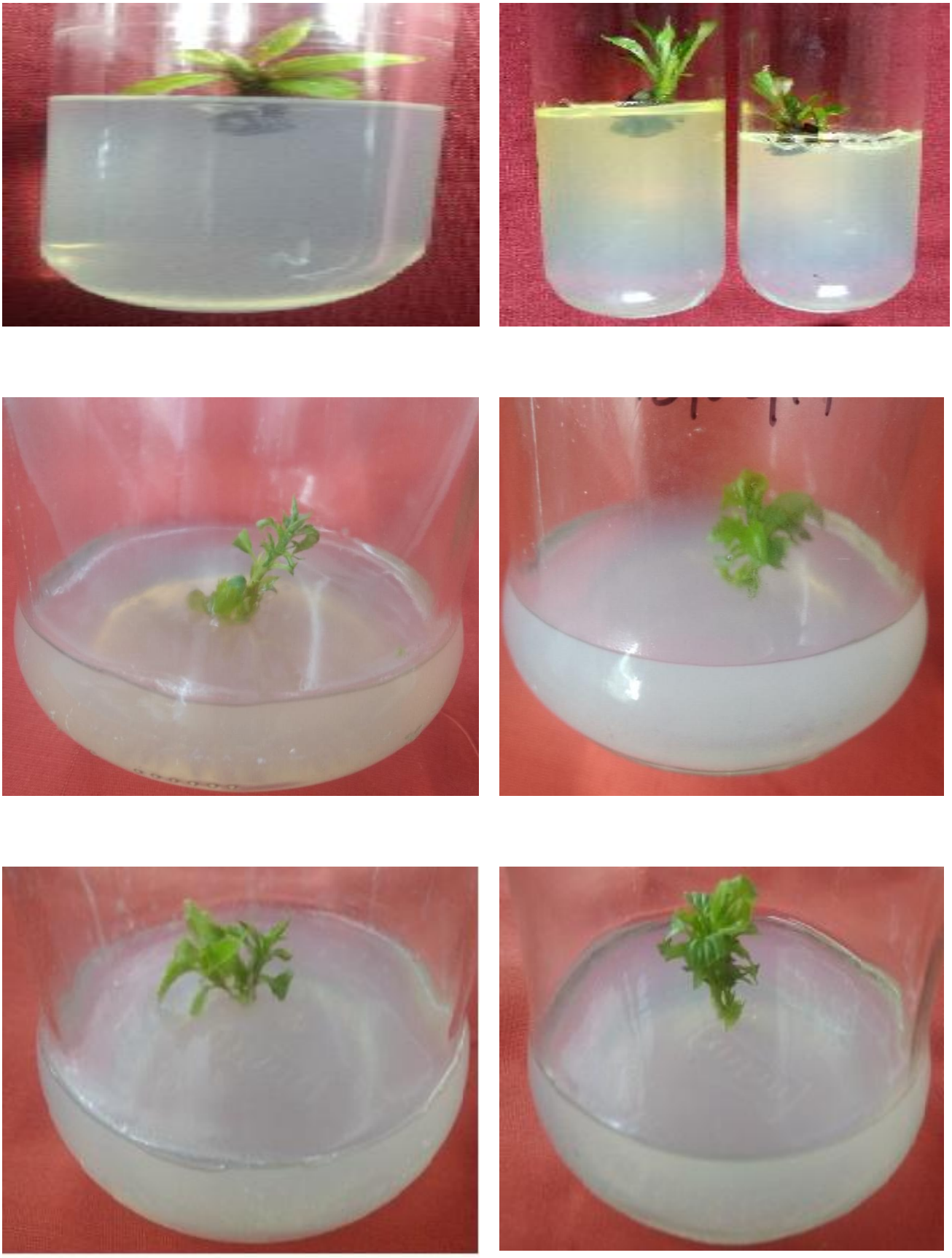


Figure 4.15: Effect of media composition on number of days taken for culture establishment of clonal apple rootstocks

(Shoot tip) **(Nodal segment)**

Plate 1: Culture establishment of explants of clonal apple rootstock MM106



(Shoot tip) **(Nodal segment)**

Plate 2: Culture establishment of explants of clonal apple rootstock MM111

segment explants followed by 31.11 per cent in AM3, while shoot tip showed (27.77%) culture establishment with AM4 followed by 26.66 per cent in AM3. However, for both explants, minimum culture establishment (0.00%) was recorded under control.

In clonal apple rootstock MM111, maximum culture establishment (41.61%) was recorded with AM4 which was statistically at par with AM3 (40.61%) and was followed by AM7 (34.45%) and AM6 (33.34%). However, minimum culture establishment (0.00%) was recorded under control. Between the two explants, nodal segment explants gave maximum culture establishment (34.59%) which was significantly higher than the shoot tip (26.48%). The interaction data revealed that nodal segment explants with AM4 media composition gave maximum culture establishment (47.88%) followed by AM3 (46.66%), while shoot tip recorded (35.33%) culture establishment followed by AM3 (34.55%). The MS basal medium recorded minimum culture establishment (0.00%) for both explants.

4.8 Effect of media composition on per cent culture establishment of clonal apple rootstocks

The data with respect to the effect of different media compositions on per cent culture establishment of clonal apple rootstocks MM106 and MM11 is presented in table 4.8 and figure 4.14. Media composition EM8 (MS + 1.0 mg/l BAP + 1.0 mg/l GA_3) gave maximum culture establishment (52.77%) in clonal apple rootstock MM106 which was statistically at par with EM2 (MS + 1.0 mg/l BAP) i.e. 51.66 per cent and was followed by 39.44 per cent in EM11 (MS + 2.0 mg/l BAP + 1.0 mg/l GA_3). The MS basal medium (without BAP and GA_3) showed minimum culture establishment (0.00%). Between the two explants (shoot tip and nodal segment), nodal segment gave maximum culture establishment (27.57%) which was significantly superior to shoot tip (23.96%). In the interaction effect, nodal segment explants under media composition EM8 gave maximum culture establishment (57.77%) followed by EM2 (56.66%), while shoot tip gave maximum culture establishment (47.77%) with EM8 followed by EM2 (46.66%). However, minimum culture establishment (0.00%) for both explants was recorded under control.

In clonal apple rootstock MM111, maximum culture establishment (68.89%) was recorded under EM8 which was statistically at par with EM2 (67.78%) and was

followed by EM11 (57.78%). However, minimum culture establishment (0.00%) was recorded under control. Between the two explants, nodal segment gave maximum culture establishment (41.94%) which was significantly higher than the shoot tip (30.83%).The interaction data revealed that nodal segment explants with EM8 gave maximum culture establishment (76.66%) followed by EM2 (75.56%), while shoot tip recorded (61.11%) culture establishment with EM8 followed by EM2 (60.00%). The MS basal medium recorded minimum culture establishment (0.00%) for both the explants.

4.9 Effect of media composition on number of days taken for culture establishment of clonal apple rootstocks

The data related to the effect of media composition on number of days taken for culture establishment of clonal apple rootstocks MM106 and MM111 is presented in table 4.9 and figure 4.15. The minimum number of days taken for culture establishment (29.32) in clonal apple rootstock MM106 was recorded with media composition EM8 (MS + 1.0 mg/l BAP + 1.0 mg/l GA_3) which was statistically at par with 29.87 days with EM2 (MS + 1.0 mg/l BAP) and was followed by 30.52 days with EM11 (MS + 2.0 mg/l BAP + 1.0 mg/l GA_3). The maximum number of days (38.32) taken for culture establishment was recorded under EM16 (MS + 3.0 mg/l BAP + 3.0 mg/l GA_3). Between the two explants (shoot tip and nodal segment), nodal segment explants took minimum number of days (30.52) for culture establishment which was significantly lower than the shoot tip (36.46). In the interaction effect, nodal segment explants under EM8 media composition took minimum number of days (26.33) for culture establishment followed by EM2 (26.47 days), while shoot tip took (32.20 days) followed by 33.27 days in EM2. However, maximum number of days taken for culture establishment (35.33 and 41.30) in nodal segment and shoot tip, respectively was recorded under EM16.

In clonal apple rootstock MM111, minimum number of days (28.80) taken for culture establishment was recorded under EM8 which was statistically at par with EM2 (28.87 days) and was followed by EM8 (30.43 days). However, maximum number of days (38.32) taken for culture establishment was recorded with EM16. Between the two explants, minimum number of days (29.98) taken for culture establishment was recorded by explants of nodal segment with EM8 which was

Table 4.10: Effect of media composition on number of days taken for shoot proliferation of clonal apple rootstocks

Media Code	Treatment	MM106			MM111		
		Shoot tip	Nodal segment	Mean	Shoot tip	Nodal segment	Mean
MM1	Control: MS (Basal)	-	-	-	-	-	-
MM2	MS + BAP (1.0 mg/l)	53.00	46.56	49.78	51.44	45.00	48.22
MM3	MS + BAP (2.0 mg/l)	55.33	49.13	52.23	53.77	47.57	50.67
MM4	MS + BAP (3.0 mg/l)	57.33	51.13	54.23	55.77	49.57	52.67
MM5	MS + GA_3 (1.0 mg/l)	56.00	49.70	52.85	54.44	48.14	51.29
MM6	MS + GA_3 (2.0 mg/l)	58.00	51.76	54.88	56.44	50.20	53.32
MM7	MS + GA_3 (3.0 mg/l)	60.40	53.99	57.19	58.84	52.43	55.63
MM8	MS + BAP (1.0 mg/l) + GA_3 (1.0 mg/l)	52.40	46.26	49.33	50.84	44.70	47.77
MM9	MS + BAP (1.0 mg/l) + GA_3 (2.0 mg/l)	58.23	51.93	55.08	56.67	50.37	53.52
MM10	MS + BAP (1.0 mg/l) + GA_3 (3.0 mg/l)	60.77	54.23	57.50	59.21	52.67	55.94
MM11	MS + BAP (2.0 mg/l) + GA_3 (1.0 mg/l)	54.90	48.66	51.78	53.34	47.10	50.22
MM12	MS + BAP (2.0 mg/l) + GA_3 (2.0 mg/l)	60.93	52.13	56.53	59.37	50.57	54.97
MM13	MS + BAP (2.0 mg/l) + GA_3 (3.0mg/l)	61.23	54.66	57.95	59.67	53.10	56.39
MM14	MS + BAP (3.0 mg/l) + GA_3 (1.0 mg/l)	55.80	49.56	52.68	54.24	48.00	51.12
MM15	MS + BAP (3.0 mg/l) + GA_3 (2.0 mg/l)	61.36	55.23	58.30	59.80	53.67	56.74
MM16	MS + BAP (3.0 mg/l) + GA_3 (3.0 mg/l)	61.47	55.67	58.57	60.15	54.11	57.13
	Mean	57.81	51.37		56.27	49.81	
	$CD_{(0.05)}$	Explant (E) = 0.20 Media (M) = 0.54 E × M = 0.77			Explant (E) = 0.17 Media (M) = 0.48 E × M = 0.69		

*NAA @ 0.2% was added to each treatment

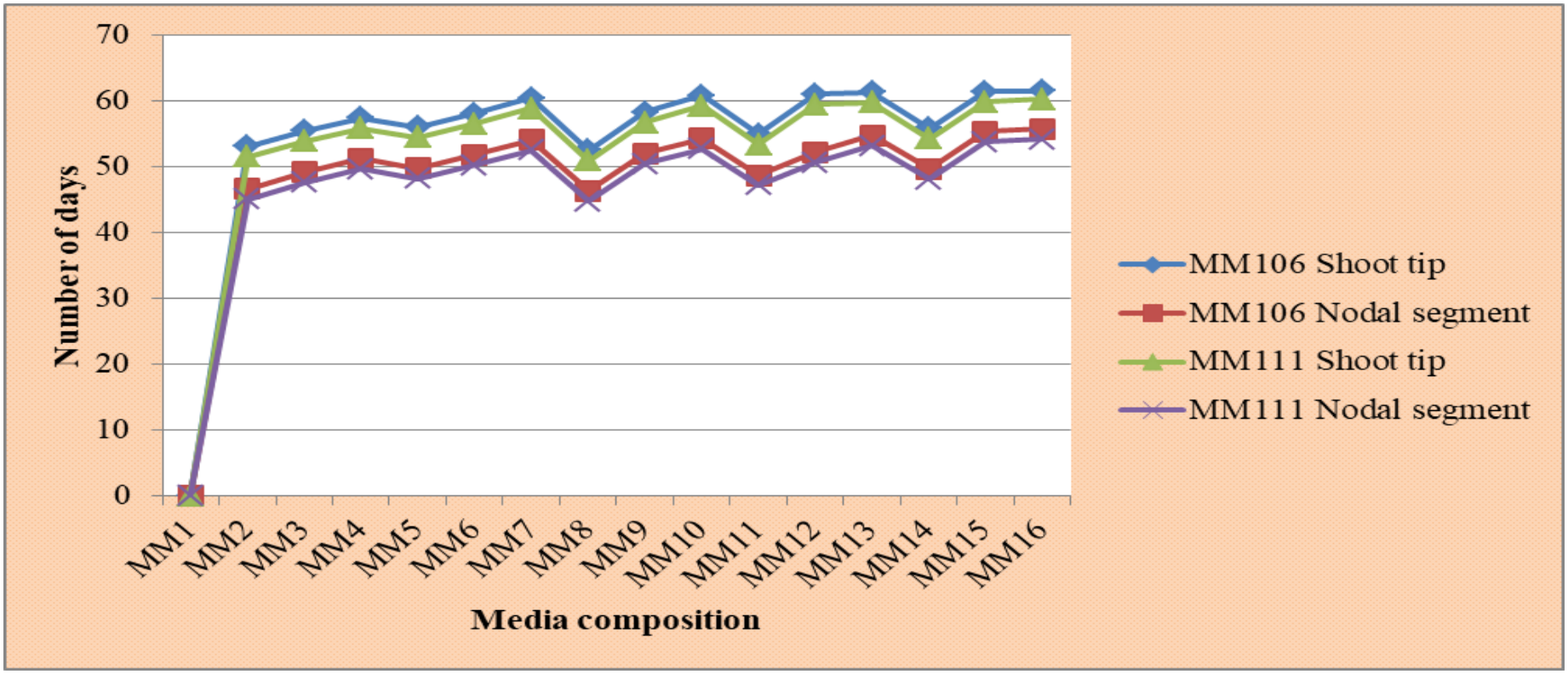


Figure 4.16: Effect of media composition on number of days taken for shoot proliferation of clonal apple rootstocks

Table 4.11: Effect of mediacomposition on number of shoots per explant of clonal apple rootstocks

Media Code	Treatment	MM106			MM111		
		Shoot tip	Nodal segment	Mean	Shoot tip	Nodal segment	Mean
MM1	Control: MS (Basal)	-	-	-	-	-	-
MM2	MS + BAP (1.0 mg/l)	2.30	4.66	3.48	2.33	5.33	3.83
MM3	MS + BAP (2.0 mg/l)	1.66	4.00	2.83	2.00	4.66	3.33
MM4	MS + BAP (3.0 mg/l)	1.30	2.66	1.98	1.33	3.33	2.33
MM5	MS + GA_3 (1.0 mg/l)	1.33	3.66	2.49	1.66	4.33	3.00
MM6	MS + GA_3 (2.0 mg/l)	1.26	2.33	1.80	1.26	3.00	2.13
MM7	MS + GA_3 (3.0 mg/l)	1.00	2.00	1.50	1.00	2.66	1.83
MM8	MS + BAP (1.0 mg/l) + GA_3 (1.0 mg/l)	2.40	4.76	3.58	2.43	5.43	3.93
MM9	MS + BAP (1.0 mg/l) + GA_3 (2.0 mg/l)	1.16	2.20	1.68	1.16	2.86	2.01
MM10	MS + BAP (1.0 mg/l) + GA_3 (3.0 mg/l)	0.76	1.93	1.35	0.87	2.60	1.73
MM11	MS + BAP (2.0 mg/l) + GA_3 (1.0 mg/l)	1.76	4.13	2.95	2.13	4.76	3.45
MM12	MS + BAP (2.0 mg/l) + GA_3 (2.0 mg/l)	1.13	2.00	1.57	1.13	2.73	1.93
MM13	MS + BAP (2.0 mg/l) + GA_3 (3.0 mg/l)	0.66	1.66	1.16	0.66	2.33	1.50
MM14	MS + BAP (3.0 mg/l) + GA_3 (1.0 mg/l)	1.43	3.76	2.60	1.73	4.43	3.08
MM15	MS + BAP (3.0 mg/l) + GA_3 (2.0 mg/l)	0.46	1.60	1.03	0.53	2.26	1.40
MM16	MS + BAP (3.0 mg/l) + GA_3 (3.0 mg/l)	0.33	1.00	0.67	0.33	1.66	1.00
	Mean	1.26	2.83		1.37	3.49	
	$CD_{(0.05)}$	Explant (E) = 0.50 Media (M) = 0.13 E × M = 0.19			Explant (E) = 0.05 Media (M) = 0.15 E × M = 0.21		

*NAA @ 0.2% was added to each treatment

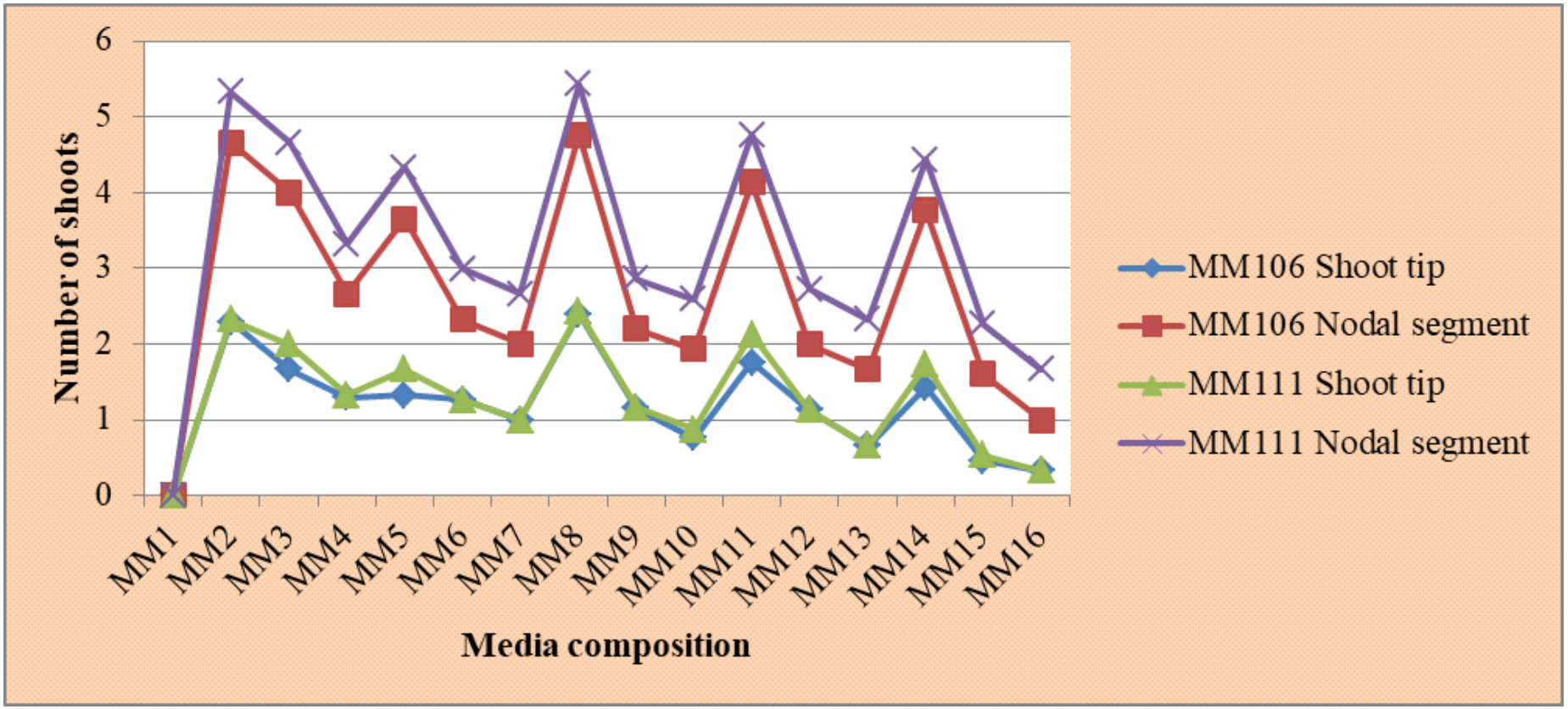


Figure 4.17: Effect of media composition on number of shoots per explant of clonal apple rootstocks

Table 4.12: Effect of mediacomposition on length of the longest shoot (cm) of clonal apple rootstocks

Media Code	Treatment	MM106			MM111		
		Shoot tip	Nodal segment	Mean	Shoot tip	Nodal segment	Mean
MM1	Control: MS (Basal)	-	-	-	-	-	-
MM2	MS + BAP (1.0 mg/l)	3.10	3.25	3.18	3.11	3.30	3.21
MM3	MS + BAP (2.0 mg/l)	2.75	3.11	2.93	2.82	3.15	2.98
MM4	MS + BAP (3.0 mg/l)	2.48	2.82	2.65	2.54	2.87	2.71
MM5	MS + GA_3 (1.0 mg/l)	2.72	2.89	2.81	2.75	2.93	2.84
MM6	MS + GA_3 (2.0 mg/l)	2.38	2.72	2.55	2.44	2.77	2.60
MM7	MS + GA_3 (3.0 mg/l)	2.19	2.53	2.36	2.24	2.57	2.41
MM8	MS + BAP (1.0 mg/l) + GA_3 (1.0 mg/l)	3.19	3.42	3.31	3.21	3.47	3.34
MM9	MS + BAP (1.0 mg/l) + GA_3 (2.0 mg/l)	2.29	2.63	2.46	2.34	2.67	2.51
MM10	MS + BAP (1.0 mg/l) + GA_3 (3.0 mg/l)	2.10	2.42	2.26	2.14	2.47	2.31
MM11	MS + BAP (2.0 mg/l) + GA_3 (1.0 mg/l)	2.93	3.16	3.05	2.96	3.20	3.08
MM12	MS + BAP (2.0 mg/l) + GA_3 (2.0 mg/l)	2.12	2.46	2.29	2.18	2.51	2.35
MM13	MS + BAP (2.0 mg/l) + GA_3 (3.0 mg/l)	1.92	2.27	2.10	1.98	2.31	2.15
MM14	MS + BAP (3.0 mg/l) + GA_3 (1.0 mg/l)	2.80	2.93	2.86	2.83	2.98	2.90
MM15	MS + BAP (3.0 mg/l) + GA_3 (2.0 mg/l)	1.87	2.21	2.04	1.92	2.25	2.09
MM16	MS + BAP (3.0 mg/l) + GA_3 (3.0 mg/l)	1.77	2.10	1.94	1.82	2.15	1.99
	Mean	2.44	2.73		2.49	2.77	
	$CD_{(0.05)}$	Explant (E) = 0.01 Media (M) = 0.03 E × M = 0.04			Explant (E) = 0.02 Media (M) = 0.04 E × M = 0.06		

*NAA @ 0.2% was added to each treatment

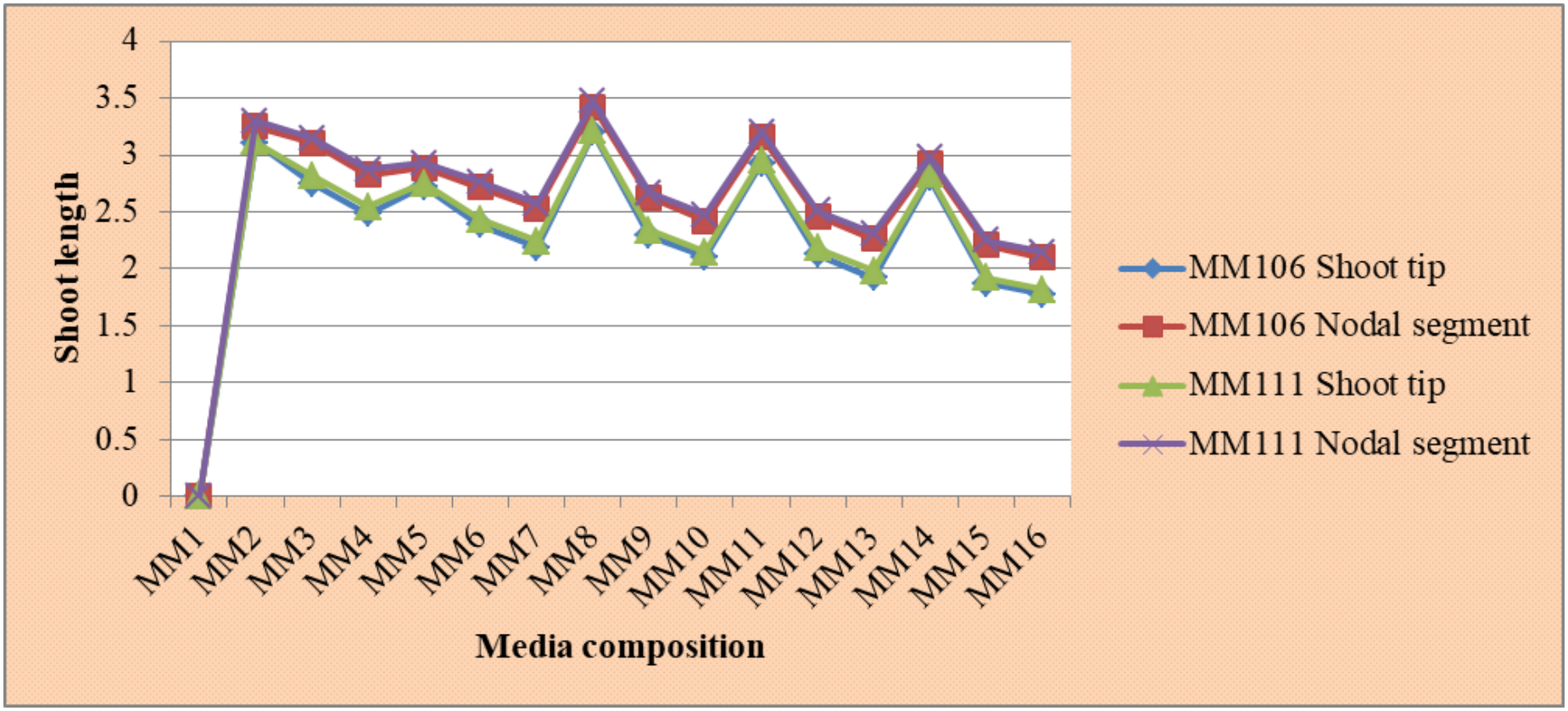


Figure 4.18: Effect of media composition on length of the longest shoot (cm) of clonal apple rootstocks

Table 4.13: Effect of media composition on number of leaves per explant of clonal apple rootstocks

Media Code	Treatment	MM106			MM111		
		Shoot tip	Nodal segment	Mean	Shoot tip	Nodal segment	Mean
MM1	Control: MS (Basal)	-	-	-	-	-	-
MM2	MS + BAP (1.0 mg/l)	9.33	10.00	9.66	10.00	10.67	10.34
MM3	MS + BAP (2.0 mg/l)	7.67	9.00	8.34	8.33	9.67	9.00
MM4	MS + BAP (3.0 mg/l)	6.33	7.33	6.83	6.67	8.00	7.33
MM5	MS + GA_3 (1.0 mg/l)	7.33	7.67	7.50	7.67	8.33	8.00
MM6	MS + GA_3 (2.0 mg/l)	5.00	7.00	6.00	6.33	7.67	7.00
MM7	MS + GA_3 (3.0 mg/l)	4.33	6.33	5.33	5.00	7.00	6.00
MM8	MS + BAP (1.0 mg/l) + GA_3 (1.0 mg/l)	9.67	10.33	10.00	10.33	11.00	10.66
MM9	MS + BAP (1.0 mg/l) + GA_3 (2.0 mg/l)	4.67	6.67	5.67	6.00	7.33	6.67
MM10	MS + BAP (1.0 mg/l) + GA_3 (3.0 mg/l)	4.00	6.00	5.00	4.33	6.67	5.50
MM11	MS + BAP (2.0 mg/l) + GA_3 (1.0 mg/l)	8.00	9.33	8.67	9.00	10.00	9.50
MM12	MS + BAP (2.0 mg/l) + GA_3 (2.0 mg/l)	4.33	6.33	5.33	5.00	7.00	6.00
MM13	MS + BAP (2.0 mg/l) + GA_3 (3.0 mg/l)	3.67	5.67	4.67	4.00	6.33	5.17
MM14	MS + BAP (3.0 mg/l) + GA_3 (1.0 mg/l)	7.67	8.00	7.84	8.00	8.67	8.34
MM15	MS + BAP (3.0 mg/l) + GA_3 (2.0 mg/l)	3.33	5.33	4.33	3.67	6.00	4.83
MM16	MS + BAP (3.0 mg/l) + GA_3 (3.0 mg/l)	3.00	4.00	3.50	3.33	4.67	4.00
	Mean	5.89	7.27		6.51	7.93	
	$CD_{(0.05)}$	Explant (E) = 0.12 Media (M) = 0.35 E × M = 0.49			Explant (E) = 0.11 Media (M) = 0.32 E × M = 0.46		

*NAA @ 0.2% was added to each treatment

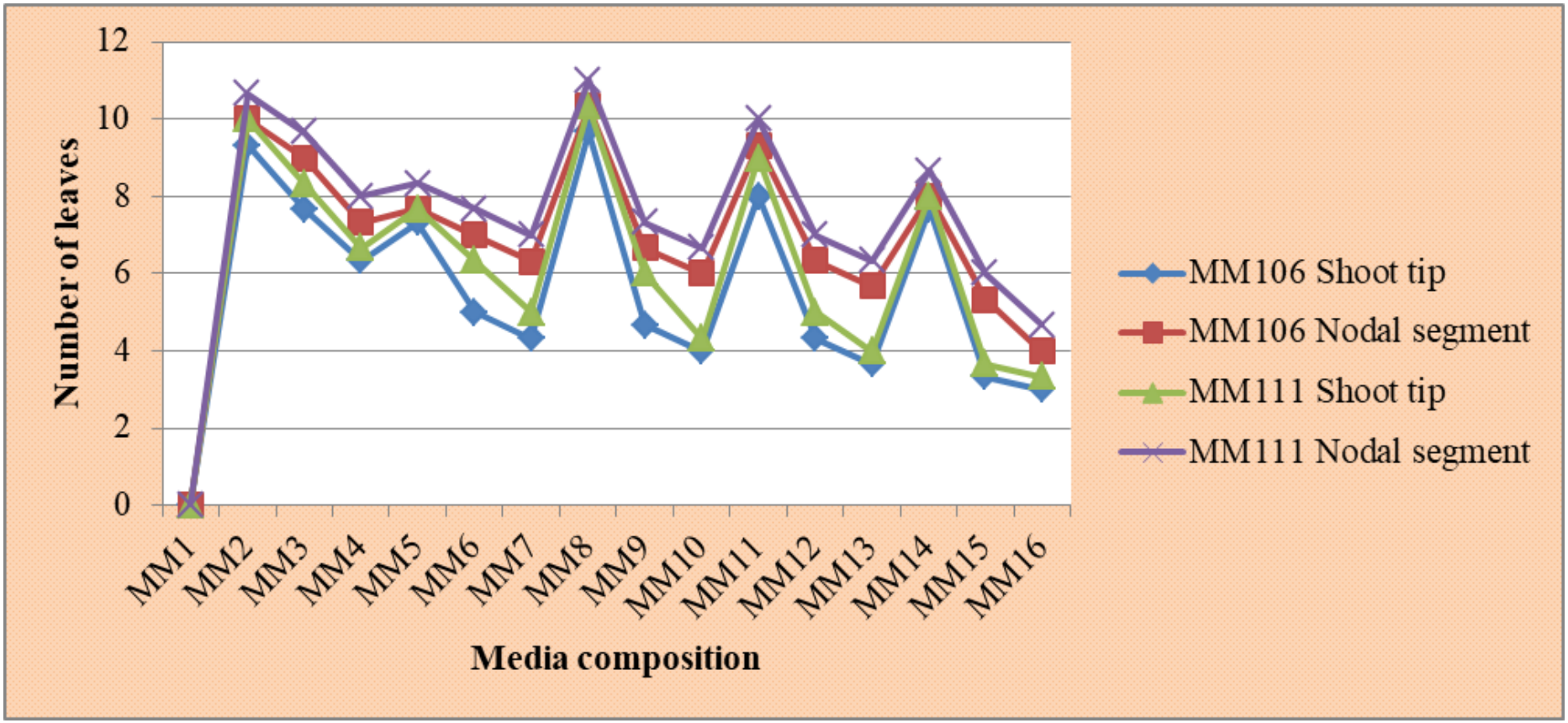
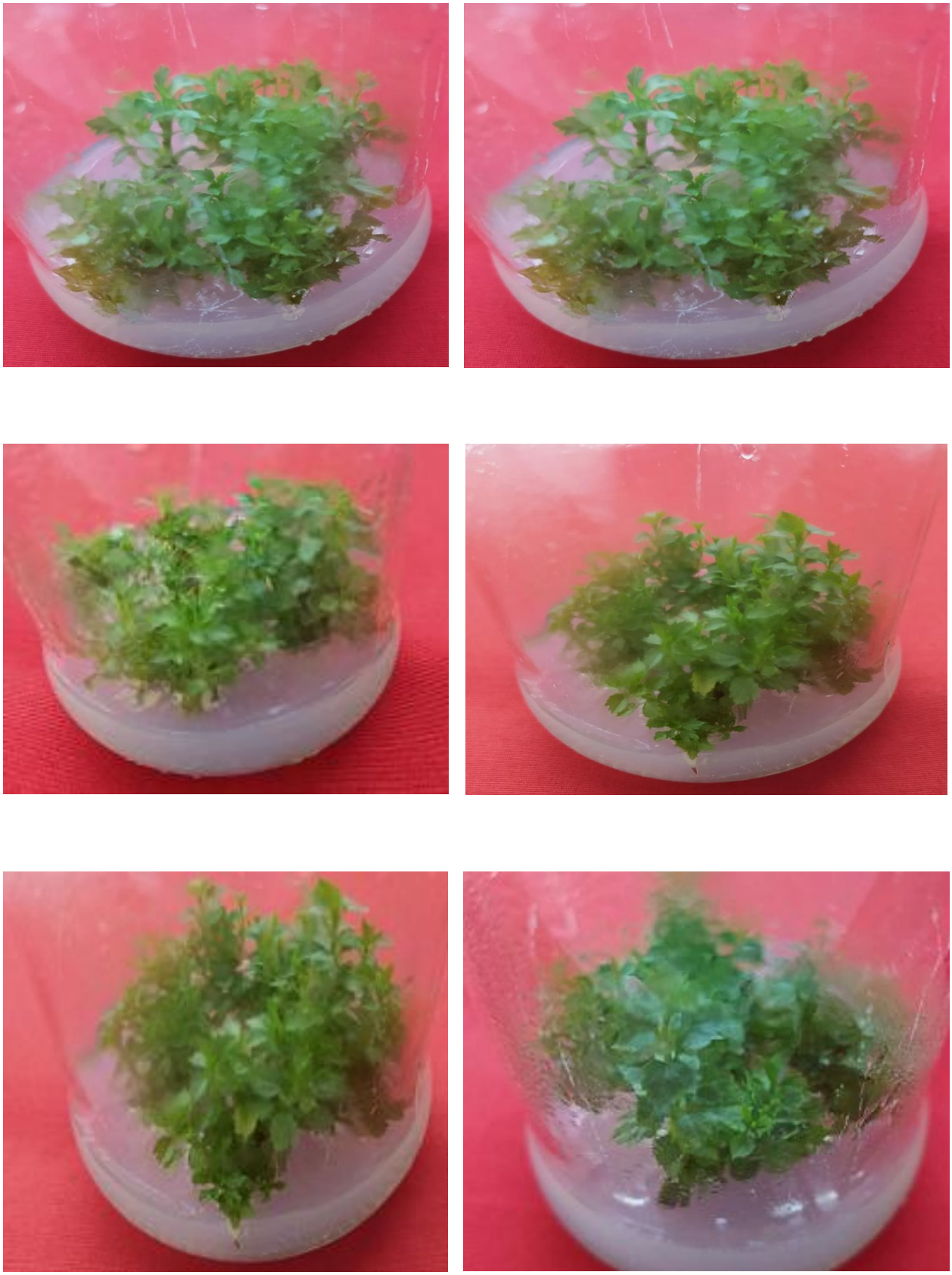
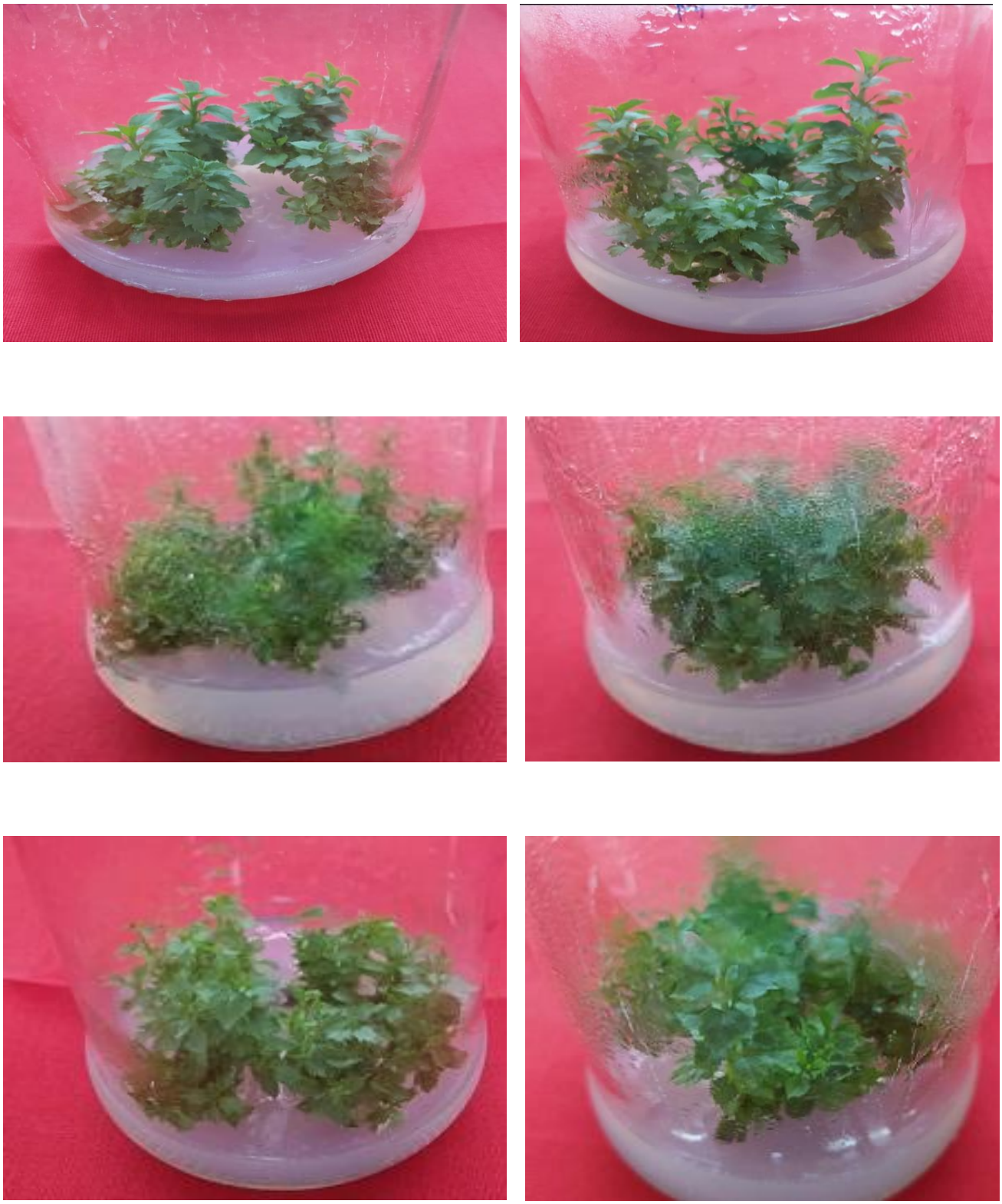


Figure 4.19: Effect of media composition on number of leaves per explant of clonal apple rootstocks



(Shoot tip) **(Nodal segment)**

Plate 3: Proliferation of explants of clonal apple rootstock MM106



(Shoot tip) **(Nodal segment)**

Plate 4: Proliferation of explants of clonal apple rootstock MM106

significantly lower than the shoot tip (35.92 days). The interaction effect indicated that nodal segment explants took minimum number of days for culture establishment (25.40) under EM8 followed by EM2 (25.47 days), whereas the media composition EM8 took minimum number of days (32.20) for culture establishment followed by EM2 (32.27 days) in shoot tip. The maximum number of days (34.13 and 40.27) taken for culture establishment in nodal segment and shoot tip, respectively was recorded under EM16.

4.10 Effect of media composition on number of days taken for shoot proliferation of clonal apple rootstocks

The data enumerated in table 4.10 and figure 4.16 revealed that different media composition showed a significant influence on number of days taken for shoot proliferation of clonal apple rootstocks MM106 and MM111. In clonal apple rootstock MM106, MM8 (MS + 1.0 mg/l BAP + 1.0 mg/l GA_3) took minimum number of days (49.33) for shoot proliferation which was statistically at par with 49.78 days in MM2 (MS + 1.0 mg/l BAP) and was followed by 51.78 days in MM11 (MS + 2.0 mg/l BAP + 1.0 mg/l GA_3). Media composition MM16 (MS + 3.0 mg/l BAP + 3.0 mg/l GA_3) took maximum number of days (58.57) for shoot proliferation. Between the two explants (shoot tip and nodal segment), nodal segment explants took minimum number of days (51.37) for shoot proliferation which was significantly lower than the shoot tip (57.81 days). The interaction data revealed that MM8 took minimum number of days (46.26) for shoot proliferation in nodal segment explants followed by MM2 (46.56 days), while in shoot tip, minimum number of days (52.40) taken for shoot proliferation was recorded with MM8 followed by MM2 (53.00 days). However, maximum number of days (55.67 and 61.47) taken for shoot proliferation in nodal segment and shoot tip, respectively was recorded under MM16.

In clonal apple rootstock MM111, MM8 took minimum number of days (47.77) for shoot proliferation, which was statistically at par with MM2 (48.22 days) and was followed by 50.22 days in MM11. The maximum number of days (57.13) taken for shoot proliferation was recorded under MM16. Between the two explants, nodal segment took minimum number of days (41.81) for shoot proliferation which was significantly lower than the shoot tip (56.27 days). The interaction data revealed that nodal segment explants under MM8 took minimum number of days (44.70) for shoot proliferation followed by MM2 (45.00 days), while in shoot tip, minimum

numbers of days (50.84) for shoot proliferation was recorded under MM8 followed by MM2 (51.44 days). However, maximum number of days (54.11and 60.15) taken for shoot proliferation in nodal segment and shoot tip, respectively was recorded under MM16.

4.11 Effect of media composition on number of shoots per explant of clonal apple rootstocks

The data with respect to the number of shoots per explants of clonal apple rootstocks MM106 and MM111 is enumerated in table 4.11 and illustrated in figure 4.17. In clonal apple rootstock MM106, media composition EM8 (MS + 1.0 mg/l BAP + 1.0 mg/l GA_3) recorded maximum number of shoots per explant (3.58) which was found to be statistically at par with 3.48 in MM2 (MS + 1.0 mg/l BAP) and was followed by 2.95 in MM11 (MS + 2.0 mg/l BAP + 1.0 mg/l GA_3). Media composition MM16 (MS + 3.0 mg/l BAP + 3.0 mg/l GA_3) recorded minimum number of shoots (0.67) per explant. Between the two different explants (shoot tip and nodal segment), nodal segment recorded maximum number of shoots per explants (2.83) which was significantly superior to the shoot tip (1.26). In the interaction effect, maximum number of shoots per explants (4.76) was recorded with media composition MM8 followed by MM2 (4.66), while shoot tip recorded (2.40) with MM8 followed by MM2 (2.30). However, minimum number of shoots per explants (0.33 and 1.00) for shoot tip and nodal segment, respectively was recorded under MM16.

The clonal apple rootstock MM111 showed maximum number of shoots (3.93) with EM8 which was statistically at par with 3.83 in MM2 and was followed by 3.45 in MM11. The minimum number of shoots (1.00) per explant was recorded under MM16. Between the two explants, nodal segment recorded maximum number of shoots (3.49) per explant which was significantly superior to the shoot tip (1.37).

In the interaction effect, maximum number of shoots (5.43) per explant was recorded with MM8 followed by MM2 (5.33), while shoot tip recorded (2.43) with MM8 followed by MM2 (2.33). However, minimum number of shoots (0.33 and 1.66) per explant of shoot tip and nodal segment, respectively was recorded under MM16.

4.12 Effect of media composition on length of the longest shoot (cm) of clonal apple rootstocks

The data enumerated in table 4.12 and figure 4.18 revealed that different media composition had a significant effect on the length of the longest shoot of clonal apple rootstocks MM106 and MM111. In clonal apple rootstock MM106, MS + 1.0 mg/l BAP + 1.0 mg/l GA_3 (EM8) recorded maximum shoot length (3.31 cm) which was significantly superior over rest of the media compositions and was followed by 3.18 cm in MM2 (MS + 1.0 mg/l BAP) and 3.05 cm in MM11 (MS + 2.0 mg/l BAP + 1.0 mg/l GA_3). The minimum shoot length (1.94 cm) was recorded with MS + 3.0 mg/l BAP + 3.0 mg/l GA_3 (MM16). Between the two explants, nodal segment recorded maximum shoot length (2.73 cm) which was significantly higher than the shoot tip (2.44 cm). The interaction study indicated that the maximum shoot length (3.42 cm) was recorded under media composition MM8 followed by 3.25 cm in MM2, while shoot tip recorded (3.19 cm) under MM8 followed by 3.10 cm in MM2. However, minimum shoot length (1.77 cm and 2.10 cm) of shoot tip and nodal segment explants, respectively was recorded under MM16.

In clonal apple rootstock MM111, media composition EM8 (MS + 1.0 mg/l BAP + 1.0 mg/l GA_3) recorded maximum shoot length (3.34 cm) which was significantly superior among all other media composition and was followed by 3.21 cm in MM2 (MS + 1.0 mg/l BAP) and 3.08 cm in MM11 (MS + 2.0 mg/l BAP + 1.0 mg/l GA_3). The minimum shoot length (1.99 cm) was recorded under MM16. Between the two different explants, nodal segment recorded maximum shoot length (2.77 cm) which was significantly superior to the shoot tip (2.49 cm). The interaction study revealed that the maximum shoot length (3.47 cm) was recorded under MM8 followed by 3.30 cm in MM2, while shoot tip recorded (3.21 cm) under MM8 followed by 3.11 cm in MM2. However, minimum shoot length (1.82 cm and 2.15 cm) in shoot tip and nodal segment, respectively was recorded under MM16.

4.13 Effect of media composition on number of leaves per explant of clonal apple rootstocks

The data presented in table 4.13 and figure 4.19 revealed that maximum number of leaves (10.00) in clonal apple rootstock MM106 was recorded under media composition MM8 (MS + 1.0 mg/l BAP + 1.0 mg/l GA_3) which was found to be

statistically at par with 9.66 in MM2 (MS + 1.0 mg/l BAP) and was followed by 8.67 in MM11 (MS + 2.0 mg/l BAP + 1.0 mg/l GA_3). The minimum number of leaves (3.50) was recorded under MM16 (MS + 3.0 mg/l BAP + 3.0 mg/l GA_3). Between the two explants, nodal segment recorded maximum number of leaves (7.27) which was also statistically superior to shoot tip (5.89). The interaction data revealed that the nodal explants recorded maximum number of leaves (10.33) with MM8 followed by 10.00 in MM2, while shoot tip recorded (9.67) with MM8 followed by 9.33 in MM2. However, minimum number of leaves (3.00 and 4.00) for shoot tip and nodal segment, respectively was recorded under MM16.

The maximum number of leaves (10.66) in clonal apple rootstock MM111 was recorded with MM8 which was statistically at par with MM2 (10.34) and was followed by MM11 (9.50). The minimum number of leaves (4.00) was recorded under MM16. Between the two different explants, nodal segment explants recorded maximum number of leaves (7.93) which was significantly higher than the shoot tip (6.51). In the interaction effect, nodal segment explants recorded maximum number of leaves (11.00) with MM8 followed by MM2 (10.67), while shoot tip recorded (10.33) with MM8 followed by MM2 (10.00). However, minimum number of leaves (3.33 and 4.67) in shoot tip and nodal segment, respectively was recorded under MM16.

4.14 Effect of media compositions on *in vitro* rooting in clonal apple rootstocks

4.14.1 Per cent cultures rooted

The data enumerated in table 4.14.1 and figure 4.20 showed that the different media composition had a significant influence on per cent cultures rooted in clonal apple rootstocks MM106 and MM111. Media composition RM6 (Half MS + 1.0 mg/l NAA) recorded 63.34 per cent cultures rooted (statistically superior over all other media compositions) and was followed by 50.00 per cent in RM3 (Half MS + 1.0 mg/l IBA), 46.67 per cent in RM9 (Half MS + 0.5 mg/l IBA + 1.0 mg/l NAA) and 45.00 per cent in RM5 (Half MS + 0.5 mg/l NAA), while minimum cultures rooted (0.00%) were recorded under control (Half MS basal). Between two clonal apple rootstock MM106 and MM111, maximum cultures rooted (34.58%) were recorded in MM111 which was significantly higher than the MM106 (28.54%). The interaction data revealed that maximum cultures rooted (66.67%) in MM111 rootstock were

Table 4.14.1:Effect of media composition on per cent cultures rooted in clonal apple rootstocks

Media Code	Treatment	MM106	MM111	Mean
RM1	Control: Half MS (Basal)	0.00 (0.00)*	0.00 (0.00)	0.00 (0.00)
RM2	Half MS + IBA (0.5 mg/l)	36.67 (41.13)	43.33 (37.17)	40.00 (39.15)
RM3	Half MS + IBA (1.0 mg/l)	46.67 (46.89)	53.33 (43.07)	50.00 (44.98)
RM4	Half MS + IBA (2.0 mg/l)	30.00 (37.23)	36.67 (33.17)	33.33 (35.20)
RM5	Half MS + NAA (0.5 mg/l)	43.33 (43.07)	46.67 (41.15)	45.00 (42.11)
RM6	Half MS + NAA (1.0 mg/l)	60.00 (54.71)	66.67 (50.74)	63.34 (52.73)
RM7	Half MS + NAA (2.0 mg/l)	33.33 (39.20)	40.00 (35.23)	36.67 (37.21)
RM8	Half MS + IBA (0.5 mg/l) + NAA (0.5 mg/l)	26.66 (35.20)	33.33 (30.99)	30.00 (33.10)
RM9	Half MS + IBA (0.5 mg/l) + NAA (1.0 mg/l)	43.33 (44.98)	50.00 (41.15)	46.67 (43.06)
RM10	Half MS + IBA (0.5 mg/l) + NAA (2.0 mg/l)	13.33 (31.01)	26.67 (21.30)	20.00 (26.19)
RM11	Half MS + IBA (1.0 mg/l) + NAA (0.5 mg/l)	26.67 (33.19)	30.00 (31.08)	28.34 (32.13)
RM12	Half MS + IBA (1.0 mg/l) + NAA (1.0 mg/l)	40.00 (43.06)	46.67 (39.21)	43.33 (41.14)
RM13	Half MS + IBA (1.0 mg/l) + NAA (2.0 mg/l)	20.00 (28.76)	23.33 (26.50)	21.67 (27.63)
RM14	Half MS + IBA (2.0 mg/l) + NAA (0.5 mg/l)	10.00 (26.50)	20.00 (18.42)	15.00 (22.46)
RM15	Half MS + IBA (2.0 mg/l) + NAA (1.0 mg/l)	20.00 (28.76)	23.33 (26.50)	21.67 (27.63)
RM16	Half MS + IBA (2.0 mg/l) + NAA (2.0 mg/l)	6.67 (21.30)	13.33 (14.96)	10.00 (18.13)
	Mean	28.54 (34.69)	34.58 (30.66)	
	$CD_{(0.05)}$	Rootstock (R) = 0.87 Media (M) = 2.47 R × M = 3.49		

*Figures presented in parenthesis are arcsine transformed values

*****Activated charcoal @ 0.2% was added to each treatment

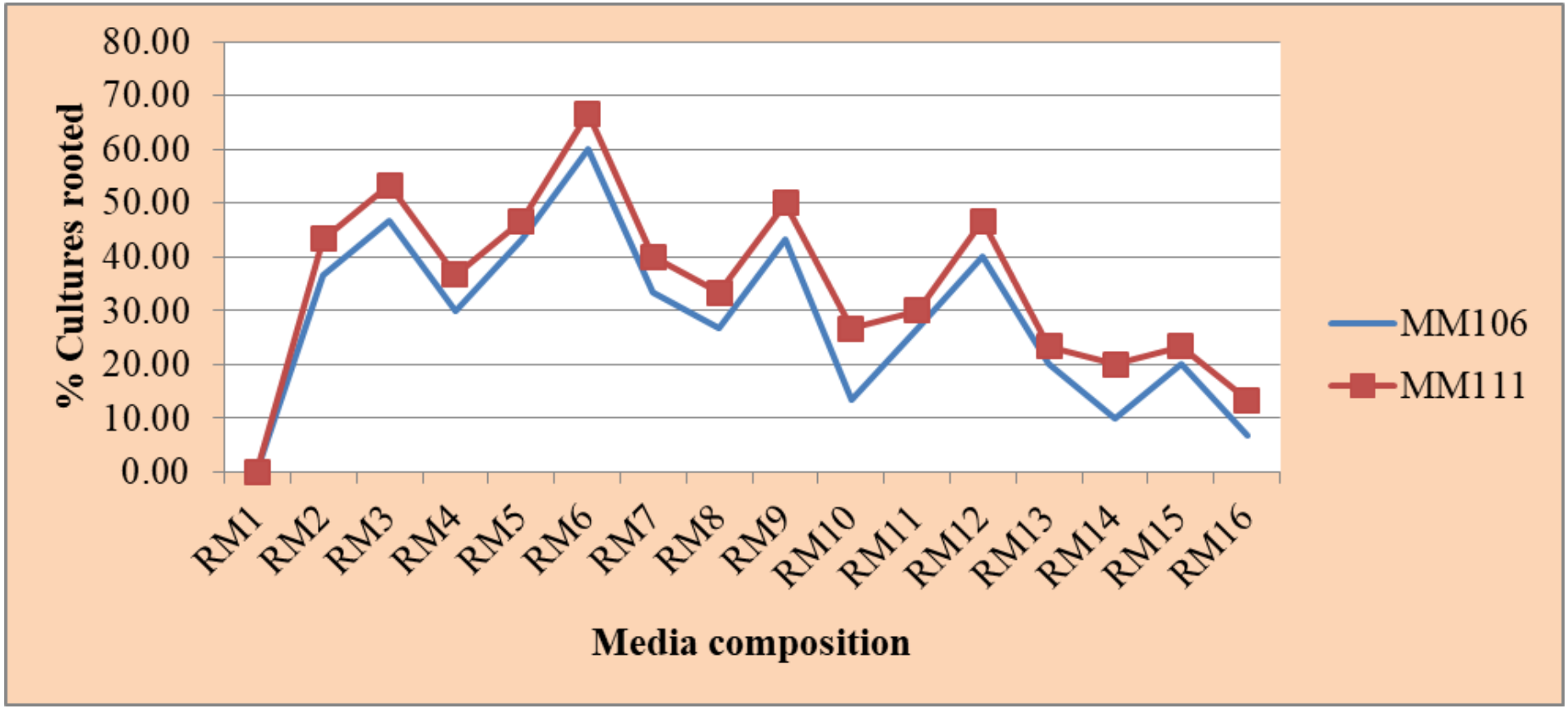


Figure 4.20: Effect of media composition on per cent cultures rooted of clonal apple rootstocks

Table 4.14.2 Effect of mediacomposition on number of days taken for root initiation of clonal apple rootstocks

Media Code	Treatment	MM106	MM111	Mean
RM1	Control: Half MS (Basal)	-	-	-
RM2	Half MS + IBA (0.5 mg/l)	16.33	15.67	16.00
RM3	Half MS + IBA (1.0 mg/l)	15.67	14.33	15.00
RM4	Half MS + IBA (2.0 mg/l)	18.33	18.00	18.17
RM5	Half MS + NAA (0.5 mg/l)	16.00	15.33	15.67
RM6	Half MS + NAA (1.0 mg/l)	14.00	13.00	13.50
RM7	Half MS + NAA (2.0 mg/l)	17.67	17.00	17.34
RM8	Half MS + IBA (0.5 mg/l) + NAA (0.5 mg/l)	14.33	13.33	13.83
RM9	Half MS + IBA (0.5 mg/l) + NAA (1.0 mg/l)	15.00	14.67	14.84
RM10	sHalf MS + IBA (0.5 mg/l) + NAA (2.0 mg/l)	18.67	15.67	17.17
RM11	Half MS + IBA (1.0 mg/l) + NAA (0.5 mg/l)	18.00	18.00	18.00
RM12	Half MS + IBA (1.0 mg/l) + NAA (1.0 mg/l)	17.67	17.33	17.50
RM13	Half MS + IBA (1.0 mg/l) + NAA (2.0 mg/l)	19.33	18.67	19.00
RM14	Half MS + IBA (2.0 mg/l) + NAA (0.5 mg/l)	19.67	19.00	19.33
RM15	Half MS + IBA (2.0 mg/l) + NAA (1.0 mg/l)	20.33	19.67	20.00
RM16	Half MS + IBA (2.0 mg/l) + NAA (2.0 mg/l)	22.00	21.33	21.66
	Mean	17.53	16.73	
	$CD_{(0.05)}$	Rootstock (R) = 0.09 Media (M) = 0.25 R × M = 0.36		

*****Activated charcoal @ 0.2% was added to each treatment

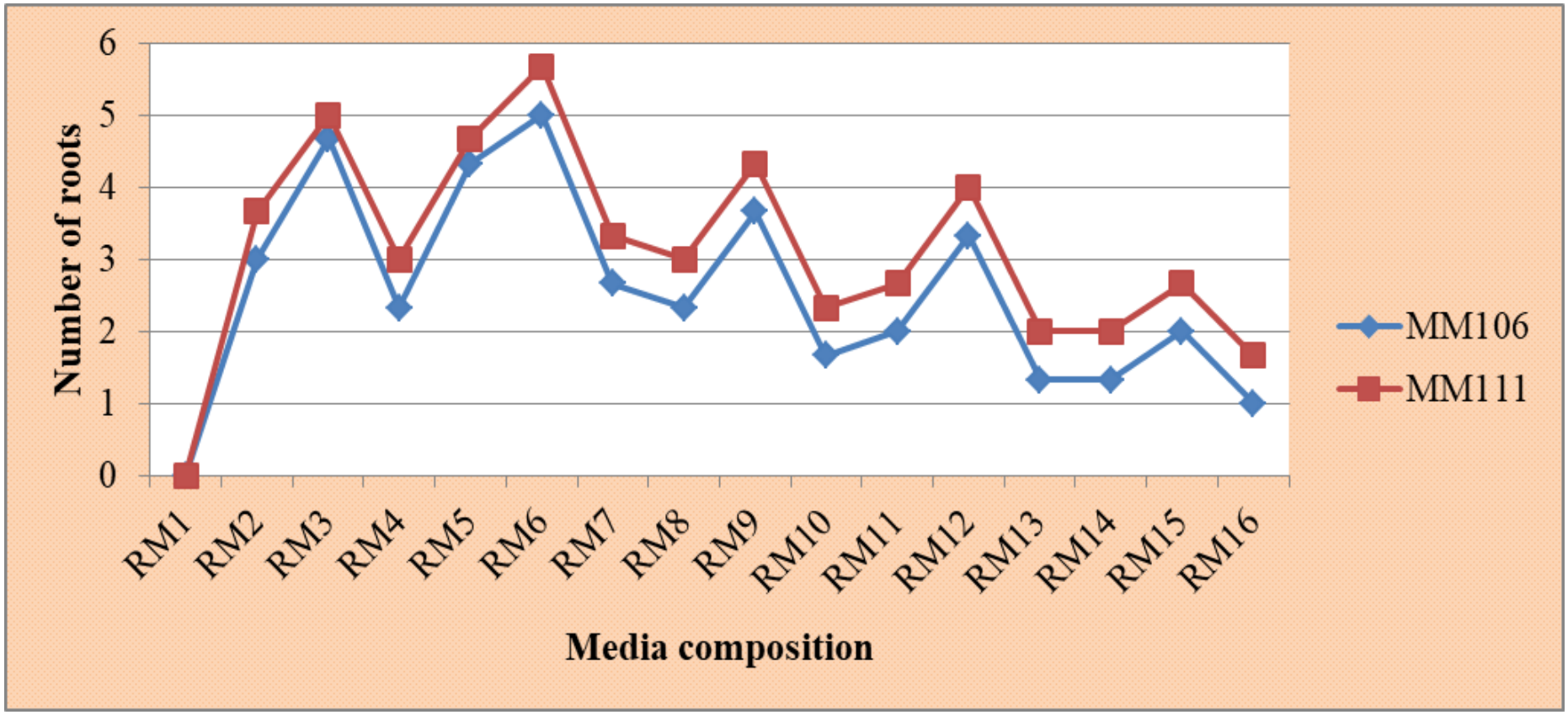


Figure 4.21: Effect of media composition on number of roots of clonal apple rootstocks

Table 4.14.3: Effect of media composition on number roots per explant of clonal apple rootstocks

Media Code	Treatment	MM106	MM111	Mean
RM1	Control: Half MS (Basal)	-	-	-
RM2	Half MS + IBA (0.5 mg/l)	3.00	3.67	3.34
RM3	Half MS + IBA (1.0 mg/l)	4.67	5.00	4.83
RM4	Half MS + IBA (2.0 mg/l)	2.33	3.00	2.67
RM5	Half MS + NAA (0.5 mg/l)	4.33	4.67	4.50
RM6	Half MS + NAA (1.0 mg/l)	5.00	5.67	5.34
RM7	Half MS + NAA (2.0 mg/l)	2.67	3.33	3.00
RM8	Half MS + IBA (0.5 mg/l) + NAA (0.5 mg/l)	2.33	3.00	2.67
RM9	Half MS + IBA (0.5 mg/l) + NAA (1.0 mg/l)	3.67	4.33	4.00
RM10	Half MS + IBA (0.5 mg/l) + NAA (2.0 mg/l)	1.67	2.33	2.00
RM11	Half MS + IBA (1.0 mg/l) + NAA (0.5 mg/l)	2.00	2.67	2.34
RM12	Half MS + IBA (1.0 mg/l) + NAA (1.0 mg/l)	3.33	4.00	3.67
RM13	Half MS + IBA (1.0 mg/l) + NAA (2.0 mg/l)	1.33	2.00	1.67
RM14	Half MS + IBA (2.0 mg/l) + NAA (0.5 mg/l)	1.33	2.00	1.67
RM15	Half MS + IBA (2.0 mg/l) + NAA (1.0 mg/l)	2.00	2.67	2.34
RM16	Half MS + IBA (2.0 mg/l) + NAA (2.0 mg/l)	1.00	1.67	1.34
	Mean	2.71	3.33	4.50
	$CD_{(0.05)}$	Rootstock (R) = 0.03 Media (M) = 0.10 R × M = 0.14		

*****Activated charcoal @ 0.2% was added to each treatment

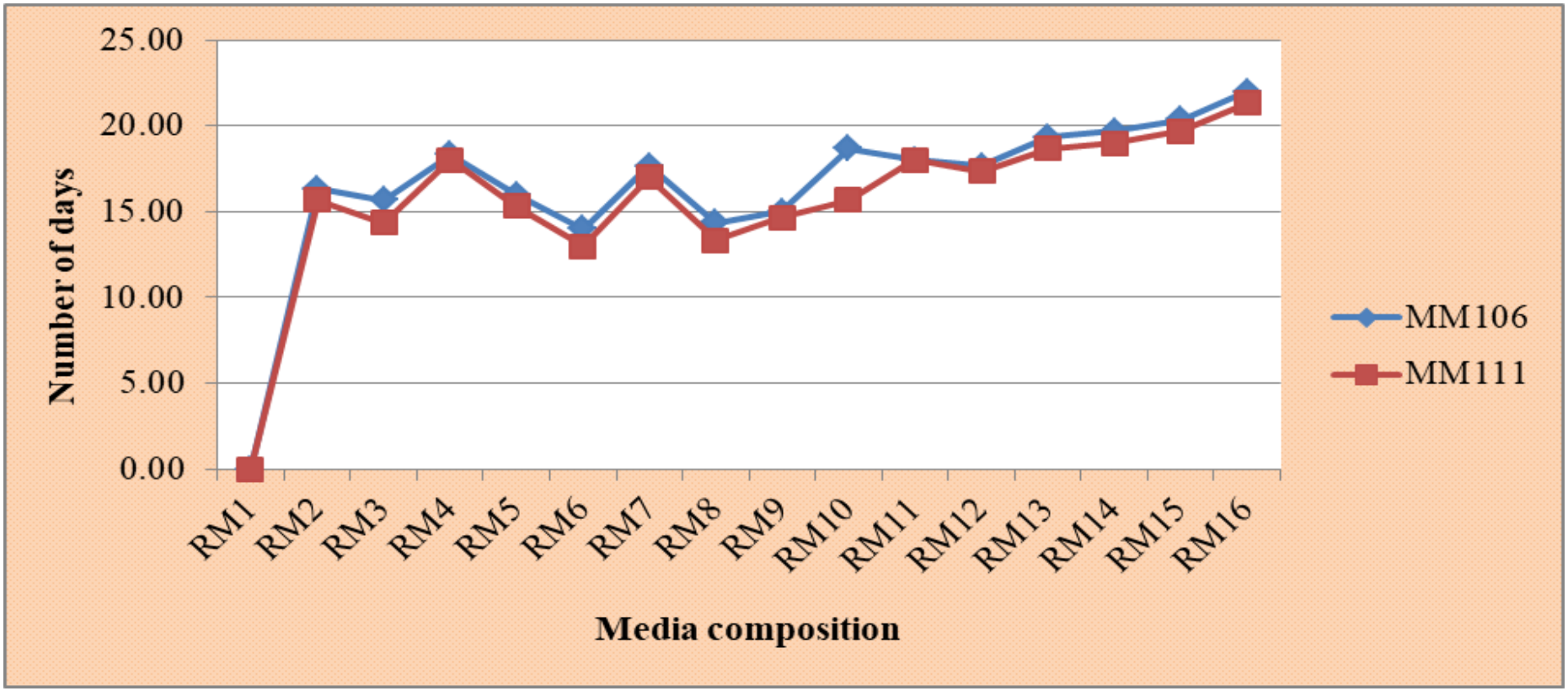


Figure 4.22: Effect of media composition on number of days taken for root initiation of clonal apple rootstocks

Table 4.14.4: Effect of mediacomposition on length of the longest root (cm) of clonal apple rootstocks

Media Code	Treatment	MM106	MM111	Mean
RM1	Control: Half MS (Basal)	-	-	-
RM2	Half MS + IBA (0.5 mg/l)	2.68	2.80	2.74
RM3	Half MS + IBA (1.0 mg/l)	3.63	3.65	3.64
RM4	Half MS + IBA (2.0 mg/l)	2.21	2.30	2.26
RM5	Half MS + NAA (0.5 mg/l)	2.79	2.86	2.83
RM6	Half MS + NAA (1.0 mg/l)	3.70	3.75	3.73
RM7	Half MS + NAA (2.0 mg/l)	2.29	2.36	2.33
RM8	Half MS + IBA (0.5 mg/l) + NAA (0.5 mg/l)	2.59	2.69	2.64
RM9	Half MS + IBA (0.5 mg/l) + NAA (1.0 mg/l)	3.33	3.35	3.34
RM10	Half MS + IBA (0.5 mg/l) + NAA (2.0 mg/l)	2.00	2.16	2.08
RM11	Half MS + IBA (1.0 mg/l) + NAA (0.5 mg/l)	2.30	2.56	2.56
RM12	Half MS + IBA (1.0 mg/l) + NAA (1.0 mg/l)	3.23	3.25	3.24
RM13	Half MS + IBA (1.0 mg/l) + NAA (2.0 mg/l)	1.87	2.00	1.93
RM14	Half MS + IBA (2.0 mg/l) + NAA (0.5 mg/l)	1.83	1.96	1.89
RM15	Half MS + IBA (2.0 mg/l) + NAA (1.0 mg/l)	2.16	2.20	2.18
RM16	Half MS + IBA (2.0 mg/l) + NAA (2.0 mg/l)	1.69	1.80	1.75
	Mean	2.57	2.65	2.83
	$CD_{(0.05)}$	Rootstock (R) = 0.02 Media (M) = 0.06 R × M = 0.08		

*****Activated charcoal @ 0.2% was added to each treatment

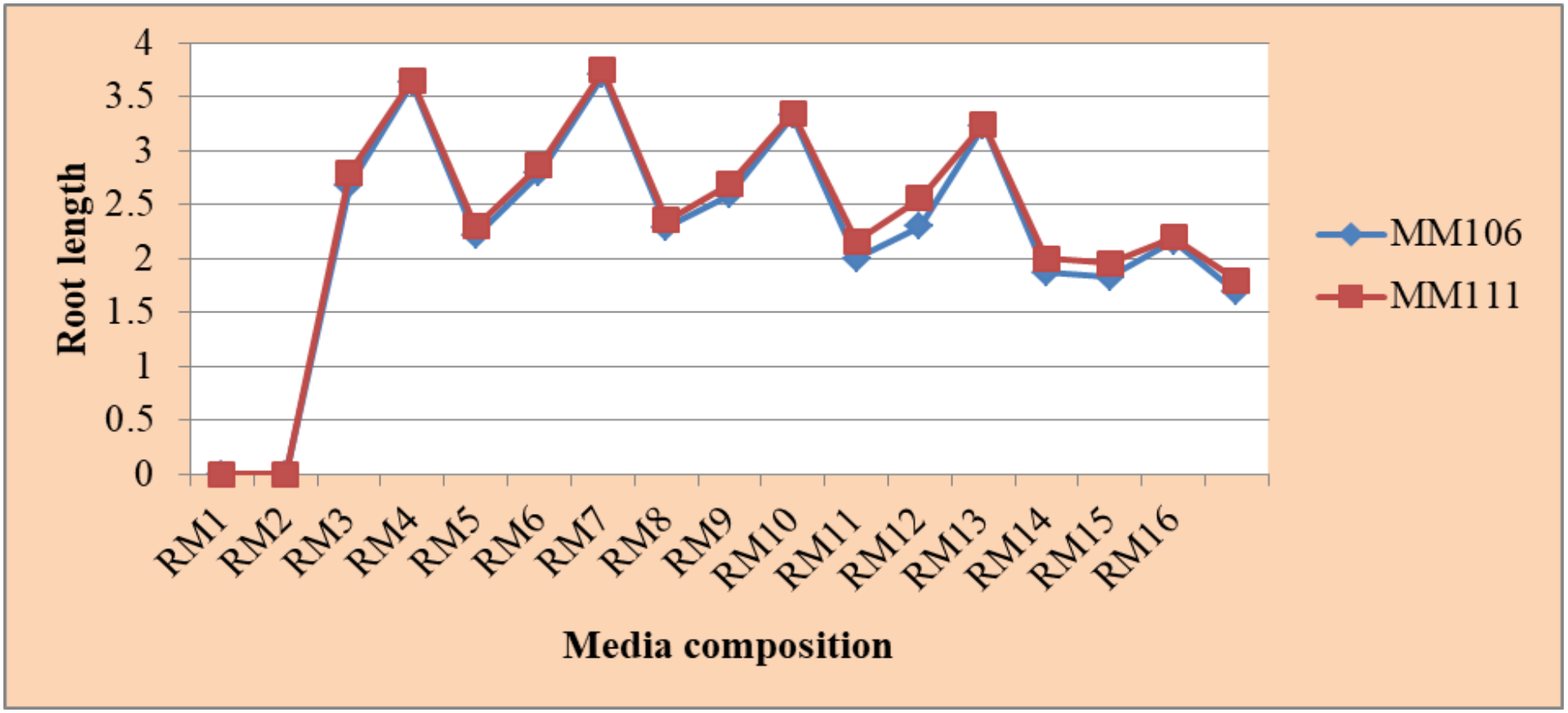


Figure 4.23: Effect of media composition on length of the longest root (cm) of clonal apple rootstocks

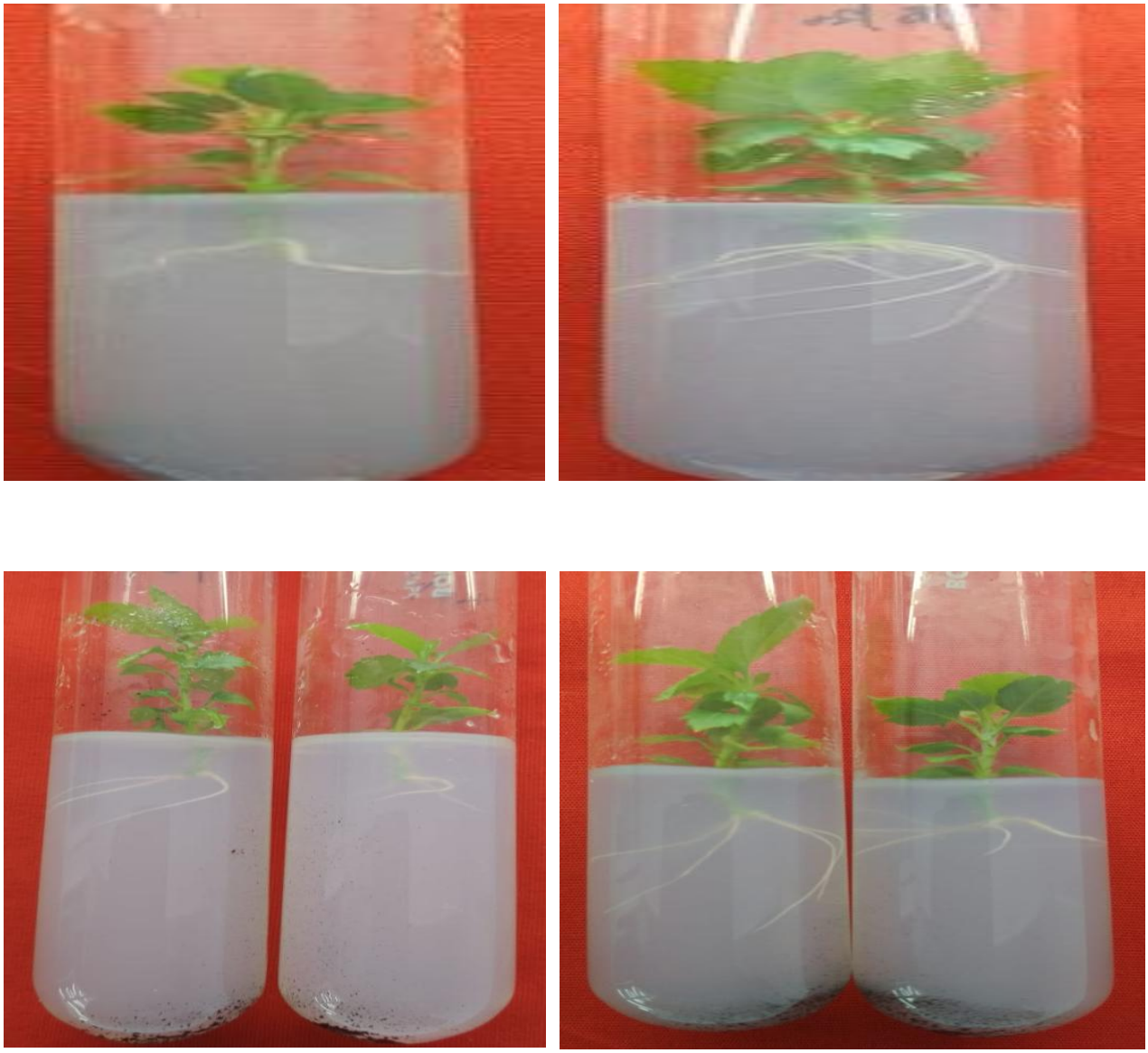


Plate 5: *In vitro* rooting of clonal apple rootstock MM106

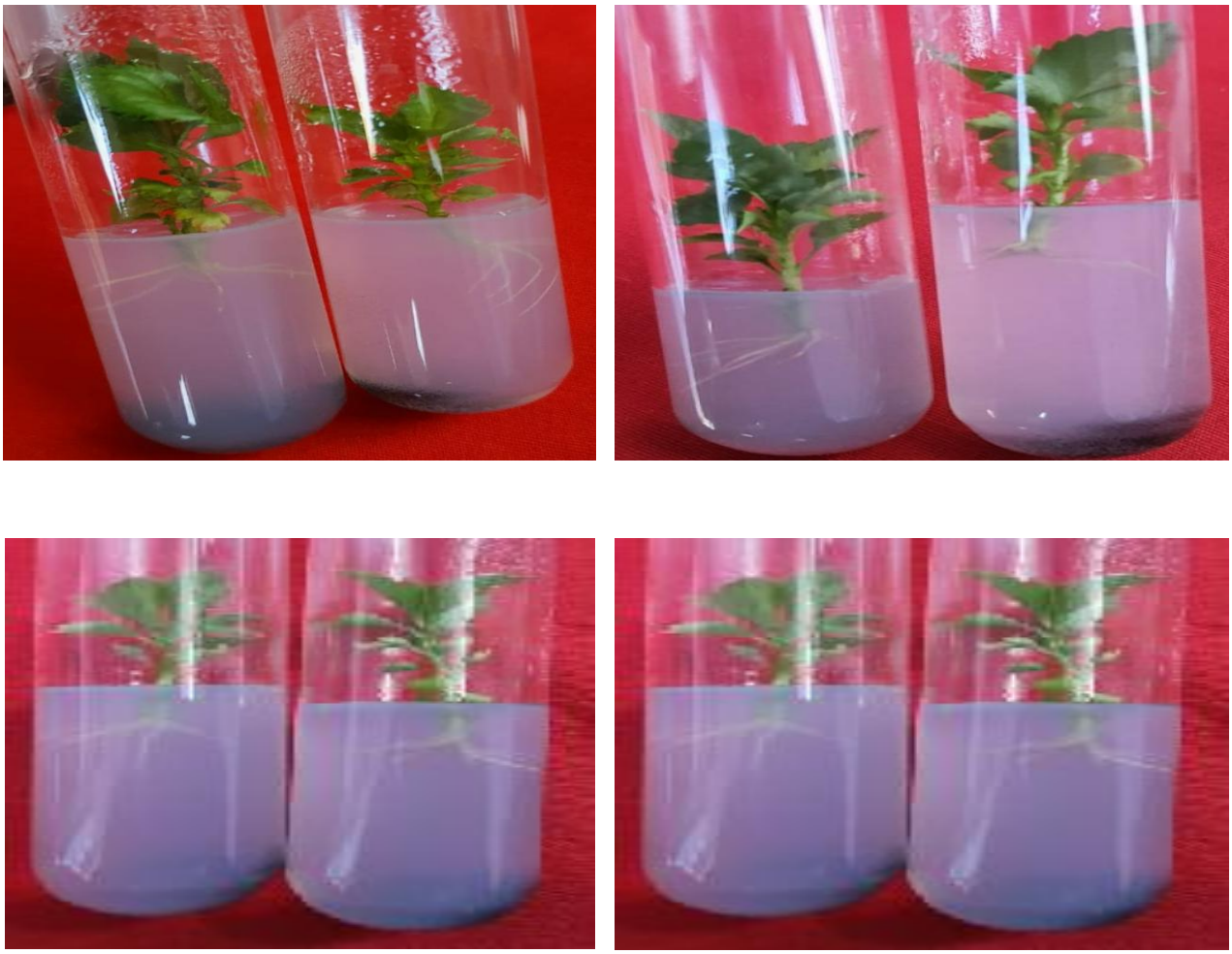


Plate 6: *In vitro* rooting of clonal apple rootstock MM111

recorded with RM6 followed by MM106 (60.00%). However, minimum cultures rooted (0.00%) were obtained under control in both the clonal rootstocks.

4.14.2 Number of days taken for root initiation

The data pertaining to the effect of media compositions on number of days taken for root initiation in clonal apple rootstocks MM106 and MM111 is presented in table 4.14.2 and figure 4.21. The minimum number of days (13.50) taken for root initiation was recorded under media composition RM6 (Half MS + NAA (1.0 mg/l) which was significantly higher over rest of the media compositions and was followed by 13.83 days in RM8 (Half MS + 1.0 mg/l NAA) and 14.84 days in RM9 (Half MS + 0.5 mg/l IBA + 1.0 mg/l NAA) and 15.00 days in RM3 (Half MS + 1.0 mg/l IBA). Between the two clonal apple rootstocks, rootstock MM111 took minimum days (16.73) for root initiation which was significantly lower than MM106 rootstock (17.53 days).

In the interaction effect, RM6 took minimum numbers of days (13.00) for root initiation in clonal apple rootstock MM111 followed by 14.00 days in MM106. However, maximum number of days (21.33 and 22.00) taken for root initiation in clonal apple rootstocks MM111 and MM106, respectively was recorded with RM16 (Half MS + 2.0 mg/l IBA + 2.0 mg/l NAA).

4.14.3 Number of roots per explant

The perusal of data presented in table 4.14.3 and figure 4.22 showed that different media composition had a significant effect on number of roots per explant of clonal apple rootstocks MM106 and MM111. RM6 (Half MS + 1.0 mg/l NAA) recorded maximum number of roots per explant (5.34) which was significantly higher over all other media compositions followed by 4.83 in RM3 (Half MS + 1.0 mg/l IBA), 4.50 in RM5 (Half MS + 0.5 mg/l NAA) and 4.00 in RM9 (Half MS + 0.5 mg/l IBA + 1.0 mg/l NAA). On an average, maximum number of roots (3.33) was observed in MM111 clonal apple rootstock which was significantly higher than MM106 rootstock (2.71).

In the interaction effect, RM6 recorded maximum number of roots per explant (5.67) in clonal apple rootstock MM111 followed by MM106 (5.00). However, minimum number of roots per explant (1.67 and 1.00) was recorded under RM16 (Half MS + 2.0 mg/l IBA + 2.0 mg/l NAA) for MM111 and MM106, respectively.

4.14.4 Length of the longest root (cm)

The data with respect to the effect of different media compositions on length of the longest root of clonal apple rootstocks MM106 and MM111 is presented in table 4.14.3 and figure 4.23 which revealed that different media composition had a significant effect on length of the longest root. The maximum root length (3.73 cm) was recorded under media composition RM6 (Half MS + 1.0 mg/l NAA) which was significantly higher than all other media compositions and was followed by 3.64 cm in RM3 (Half MS + 1.0 mg/l IBA), 3.34 cm in RM9 (Half MS + 0.5 mg/l IBA + 1.0 mg/l NAA) and 3.24 cm in RM12 (Half MS + 1.0 mg/l IBA + 1.0 mg/l NAA). Between the two different apple clonal rootstocks, MM111 rootstock showed maximum root length of 2.65 cm which was significantly higher than the MM106 rootstock (2.57 cm).

In the interaction effect, maximum root length (3.75 cm and 3.70 cm) was recorded in media composition RM6 for clonal apple rootstock MM111 and MM106, respectively. However, minimum root length (1.80 cm and 1.69 cm) was recorded under RM16 (Half MS + 2.0 mg/l IBA + 2.0 mg/l NAA) for MM111 and MM106, respectively.

4.15 Effect of soil medium on *in vitro* raised plantlets of clonal apple rootstocks

4.15.1 Per cent survival

The effect of different soil medium on per cent survival of *in vitro* raised plantlets of clonal apple rootstocks MM106 and MM111 is presented in table 4.15.1 and figure 4.24 which showed a significant effect on per cent survival. Media composition SM7 (soil + sand + vermiculite + FYM) recorded maximum survival (75.00%) which was significantly higher than all other medium compositions and was followed by 60.00 per cent with SM5 (soil + sand + vermiculite) and 50.00 per cent with SM6 (soil + sand + FYM), while minimum survival was observed under SM1 (100% sand) i.e. 0.00 per cent. Between the two clonal apple rootstocks (MM111 and MM106), MM111 rootstock gave maximum survival (43.33%) which was significantly higher than the MM106 rootstock (37.14%).

The interaction data revealed that maximum survival (76.67%) in clonal apple rootstock MM111 was recorded under SM7 followed by MM106 with SM7

Table 4.15.1: Effect of potting mixture on per cent survival of *in vitro* raised plantlets of clonal apple rootstocks

Media Code	Treatment	MM106	MM111	Mean
SM1	Sand (100%)	0.00 (0.00)*	0.00 (0.00)	0.00 (0.00)
SM2	Soil (100%)	20.00 (26.50)	26.67 (31.05)	23.33 (28.77)
SM3	Vermiculite (100%)	40.00 (39.20)	46.67 (43.06)	43.33 (41.13)
SM4	FYM (100%)	26.67 (31.05)	33.33 (35.23)	30.00 (33.14)
SM5	Soil + Sand + Vermiculite (1:1:1 v/v/v)	53.33 (46.89)	66.67 (54.71)	60.00 (44.98)
SM6	Soil + Sand + FYM (1:1:1 v/v/v)	46.67 (43.06)	53.33 (46.89)	50.00 (50.80)
SM7	Soil + Sand + Vermiculite + FYM (1:1:1:1 v/v/v/v)	73.33 (58.91)	76.67 (61.12)	75.00 (60.02)
	Mean	37.14 (35.09)	43.33 (38.87)	
	$CD_{(0.05)}$	Rootstock (R) = 1.17 Potting media (P) = 2.18 R × P = 3.09		

*Figures in parenthesis are arcsine transformed values

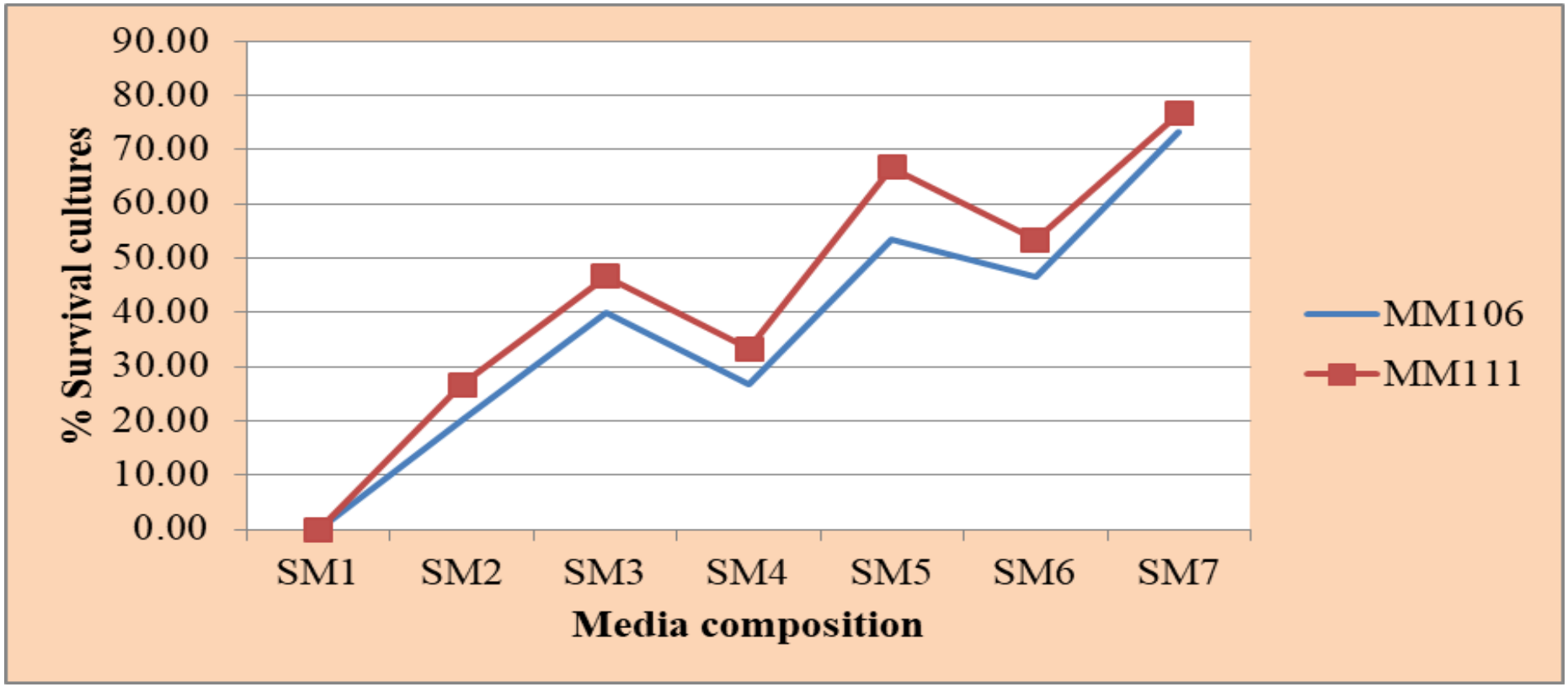


Figure 4.24: Effect of different soil mediumon per cent survival of *in vitro* raised plantlets of clonal apple rootstocks

Table 4.15.2: Effect of potting mixtures on plant height (cm) of *in vitro* raised plantlets of clonal apple rootstocks

Media Code	Treatment	MM106	MM111	Mean
SM1	Sand (100%)	-	-	-
SM2	Soil (100%)	0.52	0.60	0.56
SM3	Vermiculite (100%)	0.82	0.86	0.84
SM4	FYM (100%)	0.60	0.66	0.63
SM5	Soil + Sand + Vermiculite (1:1:1 v/v/v)	2.73	2.87	2.80
SM6	Soil + Sand + FYM (1:1:1 v/v/v)	1.94	2.13	2.03
SM7	Soil + Sand + Vermiculite + FYM (1:1:1:1 v/v/v/v)	3.23	3.30	3.26
	Mean	1.64	1.74	
	$CD_{(0.05)}$	Rootstock (R) = 0.02 Potting media (P) = 0.04 R × P = 0.05		

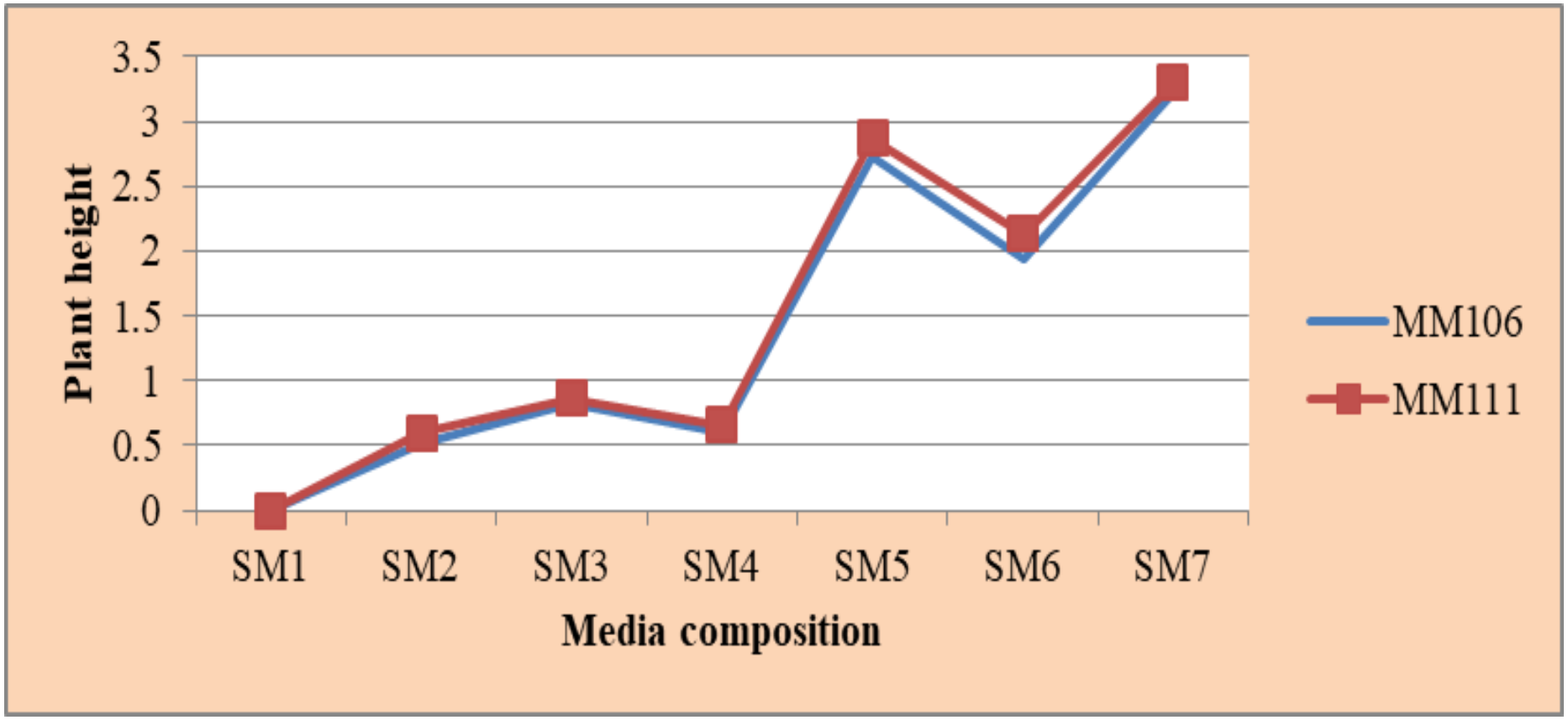


Figure 4.25: Effect of different soil medium on plant height (cm) of *in vitro* raised plantlets of clonal apple rootstocks

Table 4.15.3: Effect of potting mixture on numbers of leaves of *in vitro* raised plantlets of clonal apple rootstocks

Media Code	Treatment	MM106	MM111	Mean
SM1	Sand (100%)	-	-	-
SM2	Soil (100%)	4.00	5.00	4.50
SM3	Vermiculite (100%)	5.67	6.33	6.00
SM4	FYM (100%)	5.00	5.67	5.33
SM5	Soil + Sand + Vermiculite (1:1:1 v/v/v)	7.00	8.00	7.50
SM6	Soil + Sand + FYM (1:1:1 v/v/v)	6.00	7.00	6.50
SM7	Soil + Sand + Vermiculite + FYM (1:1:1:1 v/v/v/v)	9.00	10.00	9.50
	Mean	6.11	7.00	
	$CD_{(0.05)}$	Rootstock (R) = 0.07 Potting media (P) = 0.12 R × P = 0.17		

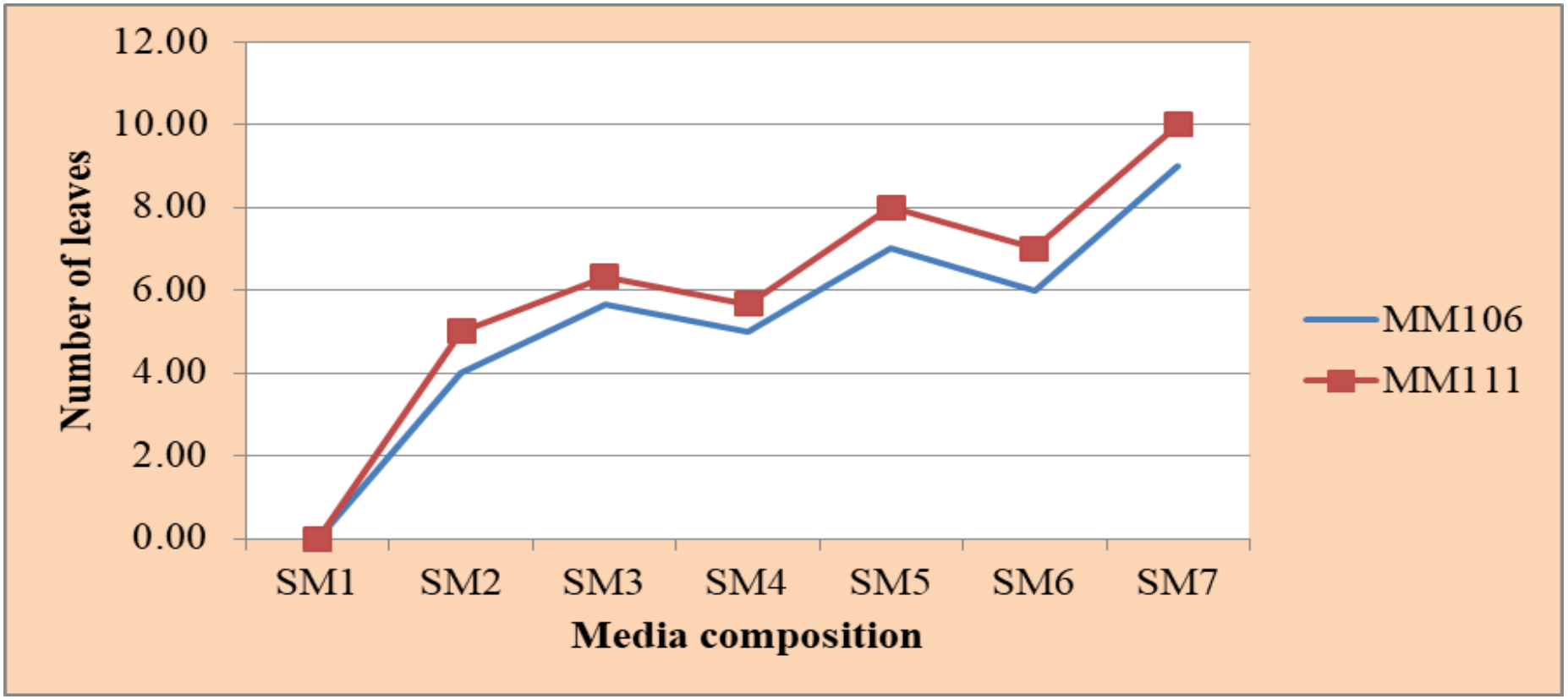


Figure 4.26: Effect of different soil medium on number of leaves of *in vitro* raised plantlets of clonal apple rootstocks

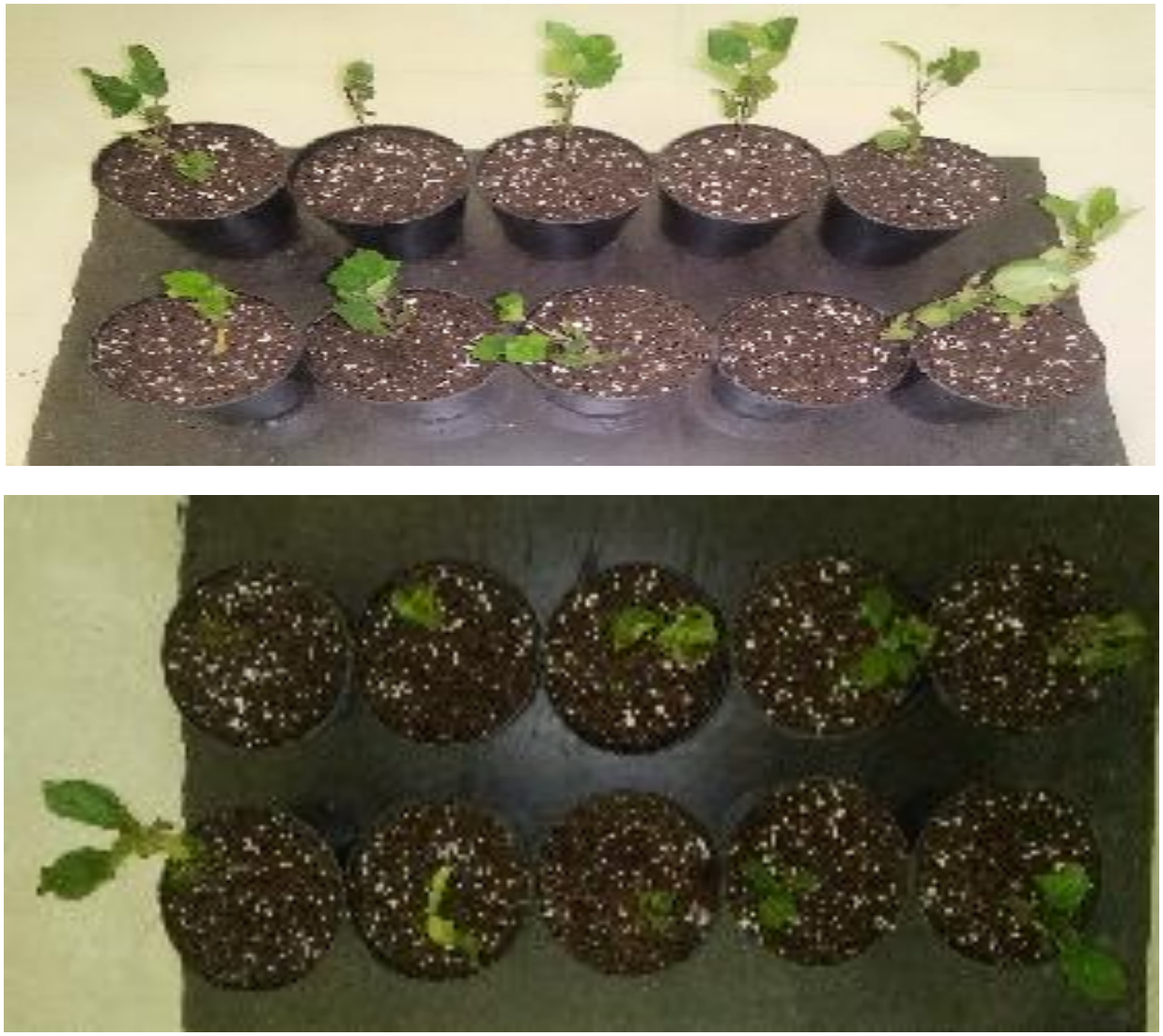


Plate 7: Hardening of *in vitro* raised plantlets of MM106

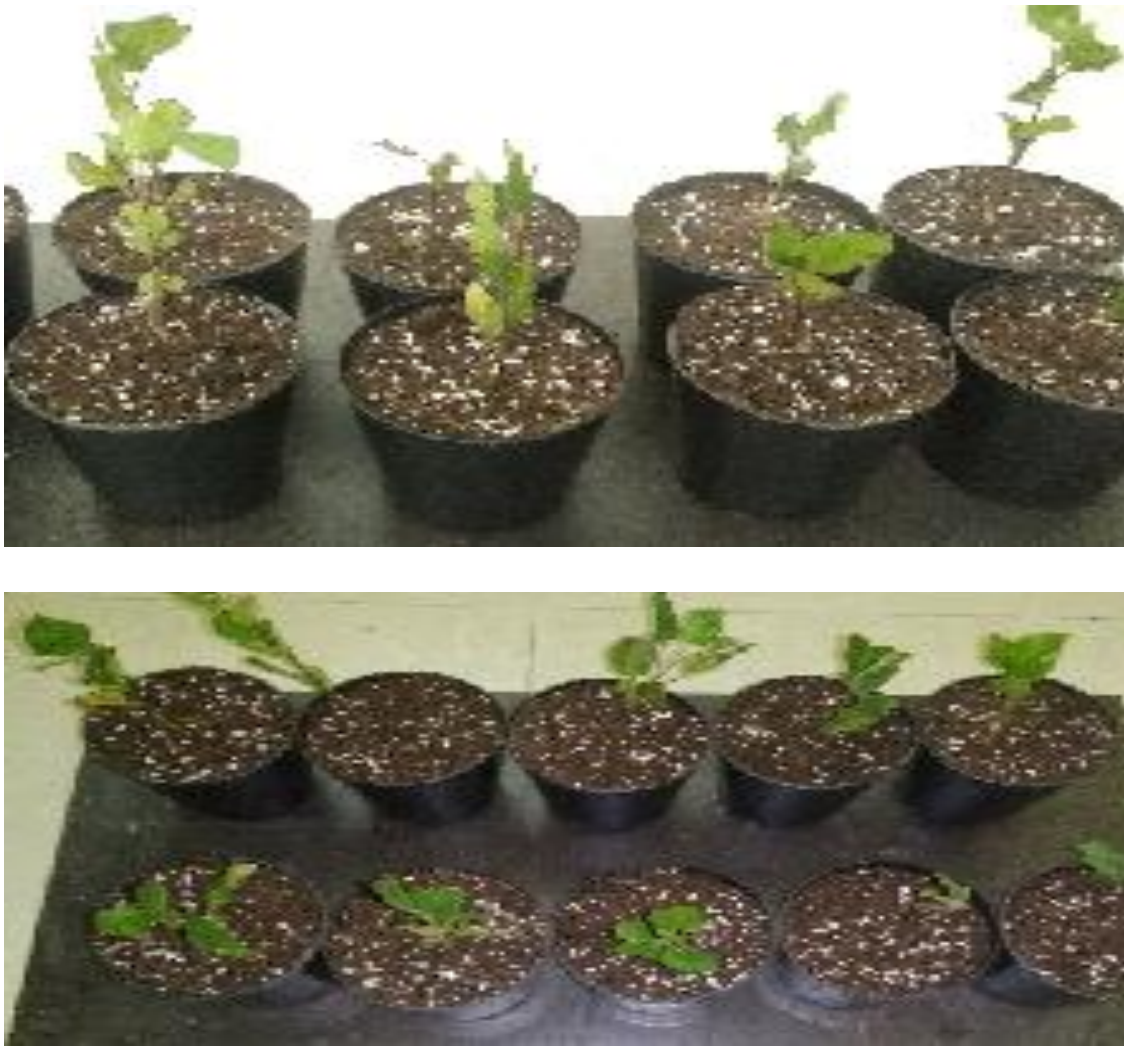


Plate 8: Hardening of *in vitro* raised plantlets of MM111

(73.33%). However, minimum survival of 0.00 per cent was recorded with 100% sand for both the clonal rootstocks.

4.15.2 Plant height (cm)

The data presented in table 4.15.2 and figure 4.25 showed a significant effect of different soil medium compositions on plant height of the clonal apple rootstocks MM106 and MM111. Maximum plant height (3.26 cm) was recorded under soil media composition SM7 (soil + sand + vermiculite + FYM) which was significantly superior over all other soil medium compositions and was followed by 2.80 cm with SM5 (soil + sand + vermiculite) and 2.03 cm with SM6 (soil + sand + FYM), whereas the minimum plant height (0.56 cm) was recorded under SM2 (100% soil). Between the two clonal apple rootstocks, MM111 rootstock recorded maximum plant height (1.74 cm) which was significantly superior to the MM106 rootstock (1.64 cm).

In the interaction effect, apple rootstock MM111 recorded maximum plant height (3.30 cm) under SM7 followed by 3.23 cm in MM106 with SM7. However, for both the apple clonal rootstocks (MM111 and MM106), minimum plant height (0.60 cm and 0.52 cm), respectively was recorded under SM2 (100% soil).

4.15.3 Number of leaves

The data enumerated in table 4.15.3 and figure 4.26 with respect to different media showed a significant effect on the number of leaves of clonal apple rootstocks MM106 and MM111. The maximum number of leaves (9.50) was observed under SM7 (soil + sand + vermiculite + FYM) which was significantly higher than all other soil media compositions and was followed by 7.50 in SM5 (soil + sand + vermiculite) and 6.50 in SM6 (soil + sand + FYM), while minimum number of leaves (4.50) was observed under SM2 (100% soil). Between the two rootstocks, clonal apple rootstock MM111 recorded maximum number of leaves (7.00) which was significantly higher than the MM106 rootstock (6.11).

In the interaction effect, clonal rootstock MM111 recoded maximum number of leaves (10.00) under SM7 followed by MM106 (9.00) under SM7. However, minimum number of leaves (5.00 and 4.00) in clonal apple rootstocks MM111 and MM106, respectively was recorded under SM2 (100% soil).

4.16 Economics of *in vitro* raised plantlets of clonal apple rootstocks

The cost of producing clonal apple rootstocks has been presented in table 4.16.1, figure 4.27 and table 4.16.2, figure 4.28 and worked out by considering the inputs and facilities of tissue culture laboratory used for maintaining about 12288 and 12864 plantlets of MM106 and MM111, respectively at a time. Based on the rate of culture establishment, rate of multiplication, rooting response of shoots and survival of the plantlets at various stages, it was found that about 12288 and 12864 plantlets of both rootstocks could be produced upto 27 weeks from 300 initial explants taken from both apple clonal rootstocks. The total cost involved was worked out based on the labor charges, cost of building, equipments, glassware, chemicals and miscellaneous items having been distributed over the years according to their potential/ durability. The cost of producing one *in vitro* grown clonal apple rootstocks MM106 and MM111 including 6 weeks hardening was found to be ₹ 44.56 and ₹ 36.95, respectively.

Table 4.16.1: Estimated cost for *in vitro* propagation of clonal apple rootstock MM106 through tissue culture on the basis of various inputs required

A. Tissue culture production phase

S. No.	Items	Cost (Rs.)
1.	Media cost (calculated on the basis of cost of MS media vials and chemical required for its supplementation at each stage)	
	(a) Stage – I (300 explants in 300 test tubes × 16 ml) = 4.80 litre	326.40
	[192 shoots in 192 test tubes (capacity 50 ml) × 16 ml] = 3.07 litre	208.76
	(b) Stage – II Cycle – A [768 shoots in 154 glass jar (capacity 150 ml) × 30 ml/ glass jars] = 4.62 litre	314.16
	Cycle – B [3072 shoots in 615 glass jars (capacity 150 ml) × 30 ml] = 18.45 litre	1254.60
	Cycle – C [12288 shoots in 2458 glass jars (capacity 150 ml) × 30 ml] = 73.74 litre	5014.32
	(C) Stage – III [9831 shoots in 9831 test tubes (capacity 50 ml) × 16 ml = 157.29 litre	10695.72
	Total media cost	**17813.96**
2.	**Glassware cost:**	
	(a) Culture vessels: (i) 3227 glass jars capacity 150 ml @ Rs. 150 per glass jar allocated over 50 monthly cycles (ii) 50 ml culture tubes (10323 @ Rs. 16.00 per tube allocated over 50 monthly cycles	9681.00 3303.36
	(b) Other glassware of media preparation (Rs. 10,000 allocated over 50 monthly cycles	200.00
	Total glassware cost	**13184.36**
3.	**Equipment and laboratory facility:**	
	(a) Equipment (Laminar air flow chamber, air conditioners, autoclaves, weight balance, pH meter, refrigerator, hot air oven etc.) Rs. 6,00,000/- allocated over 90 months	6666.66
	(b) Laboratory facility (Building with growth room) Rs. 10,00,000 allocated over 300 months	3333.33
	Total equipment and laboratory cost	**9999.99**
4.	**Wages and salary:**	
	(a) 1 Technician with total emoluments Rs. 8000 per month	48000.00
	(b) 1 Supervisor with total emoluments Rs. 10000 per month	60000.00
	Total wages and salary	**108000.00**

5.	**Miscellaneous costs:**	
	(Electricity, water etc.) Rs. 5000 per month	**30000.00**
	Total tissue culture production phase cost	**178998.31**
	Unit cost of producing clonal apple rootstock (on the basis of 4325 transplants per month	**41.38**

B. Greenhouse production phase

S. No.	Item	Cost (Rs.)
1.	Labour charges:	
	(a) 1 skilled labour @ Rs. 6000 per month	4200.00
	(b) 1 skilled greenhouse technician @ Rs. 8000 per month	5600.00
2.	Material cost:	
	(a) Pots (4325 @ Rs. 7 per pot allocated over 30 monthly cycles)	1009.16
	(b) Soil mixture (Soil + Sand + Vermiculite + FYM) all in equal proportions by v/v 100 cu ft @ Rs. 6 per cu ft.	600.00
	(c) Greenhouse implements (Rs. 10000 allocated over 20 cycles) each of 2 months duration	
3.	Greenhouse (size approximately 500 sq. ft.) facility Rs. 80000 allocated over 10 years (60 cycles) each of two month duration)	1333.33
4.	Miscellaneous cost (water, electricity, and other expenses) Rs. 1500 per month	1050.00
	Total greenhouse production phase cost	13792.49
	Unit cost during greenhouse production phase (On the basis of 4325 transplant per month)	3.18
	Total per unit cost A + B	**41.38 + 3.18**
	Total cost per tissue culturally raised plants	**44.56**

Table 4.16.2: Estimated cost for *in vitro* propagation of clonal apple rootstock MM111 through tissue culture on the basis of various inputs required

A. Tissue culture production phase

S. No.	Items	Cost (Rs.)
1.	Media cost (calculated on the basis of cost of MS media vials and chemical required for its supplementation at each stage)	
	(b) Stage – I (300 explants in 300 test tubes × 16 ml) = 4.80 litre	326.40
	[201 shoots in 201 test tubes (capacity 50 ml) × 16 ml] = 3.21 litre	218.28
	(b) Stage – II Cycle – A [804 shoots in 161 glass jar (capacity 150 ml) × 30 ml/ glass jars] = 4.83 litre	328.44
	Cycle – B [3216 shoots in 643 glass jars (capacity 150 ml) × 30 ml] = 19.29 litre	1311.72
	Cycle – C [12864 shoots in 2573 glass jars (capacity 150 ml) × 30 ml] = 77.19 litre	5248.92
	(C) Stage – III [10292 shoots in 10292 test tubes (capacity 50 ml) × 16 ml = 164.67 litre	11197.56
	Total media cost	**18631.32**
2.	**Glassware cost:**	
	(a) Culture vessels: (i) 3373 glass jars capacity 150 ml @ Rs. 150 per glass jar allocated over 50 monthly cycles (ii) 50 ml culture tubes (10793 @ Rs. 16.00 per tube allocated over 50 monthly cycles	10131.00 3453.76
	(b) Other glassware of media preparation (Rs. 10,000 allocated over 50 monthly cycles	200.00
	Total glassware cost	**13784.76**
3.	**Equipment and laboratory facility:**	
	(a) Equipment (Laminar air flow chamber, air conditioners, autoclaves, weight balance, pH meter, refrigerator, hot air oven etc.) Rs. 6,00,000/- allocated over 90 months	6666.66
	(b) Laboratory facility (Building with growth room) Rs. 10,00,000 allocated over 300 months	3333.33
	Total equipment and laboratory cost	**9999.99**
4.	**Wages and salary:**	
	(a) 1 Technician with total emoluments Rs. 8000 per month	48000.00
	(b) 1 Supervisor with total emoluments Rs. 10000 per month	60000.00
	Total wages and salary	**108000.00**
5.	**Miscellaneous costs:**	
	(Electricity, water etc.) Rs. 5000 per month	**30000.00**
	Total tissue culture production phase cost	**180416.07**

	Unit cost of producing clonal apple rootstock (on the basis of 5260 transplants per month	**34.29**

B. Greenhouse production phase

S. No.	Item	Cost (Rs.)
1.	Labour charges:	
	(a) 1 skilled labour @ Rs. 6000 per month	4200.00
	(b) 1 skilled greenhouse technician @ Rs. 8000 per month	5600.00
2.	Material cost:	
	(a) Pots (5260 @ Rs. 7 per pot allocated over 30 monthly cycles)	1227.33
	(b) Soil mixture (Soil + Sand + Vermiculite + FYM) all in equal proportions by v/v 100 cu ft @ Rs. 6 per cu ft.	600.00
	(c) Greenhouse implements (Rs. 10000 allocated over 20 cycles) each of 2 months duration	
3.	Greenhouse (size approximately 500 sq ft.) facility Rs. 80000 allocated over 10 years (60 cycles) each of two month duration)	1333.33
4.	Miscellaneous cost (water, electricity, and other expenses) Rs. 1500 per month	1050.00
	Total greenhouse production phase cost	14010.66
	Unit cost during greenhouse production phase (On the basis of 5260 transplant per month)	2.66
	Total per unit cost A + B	**34.29 + 2.66**
	Total cost per tissue culturally raised plants	**36.95**

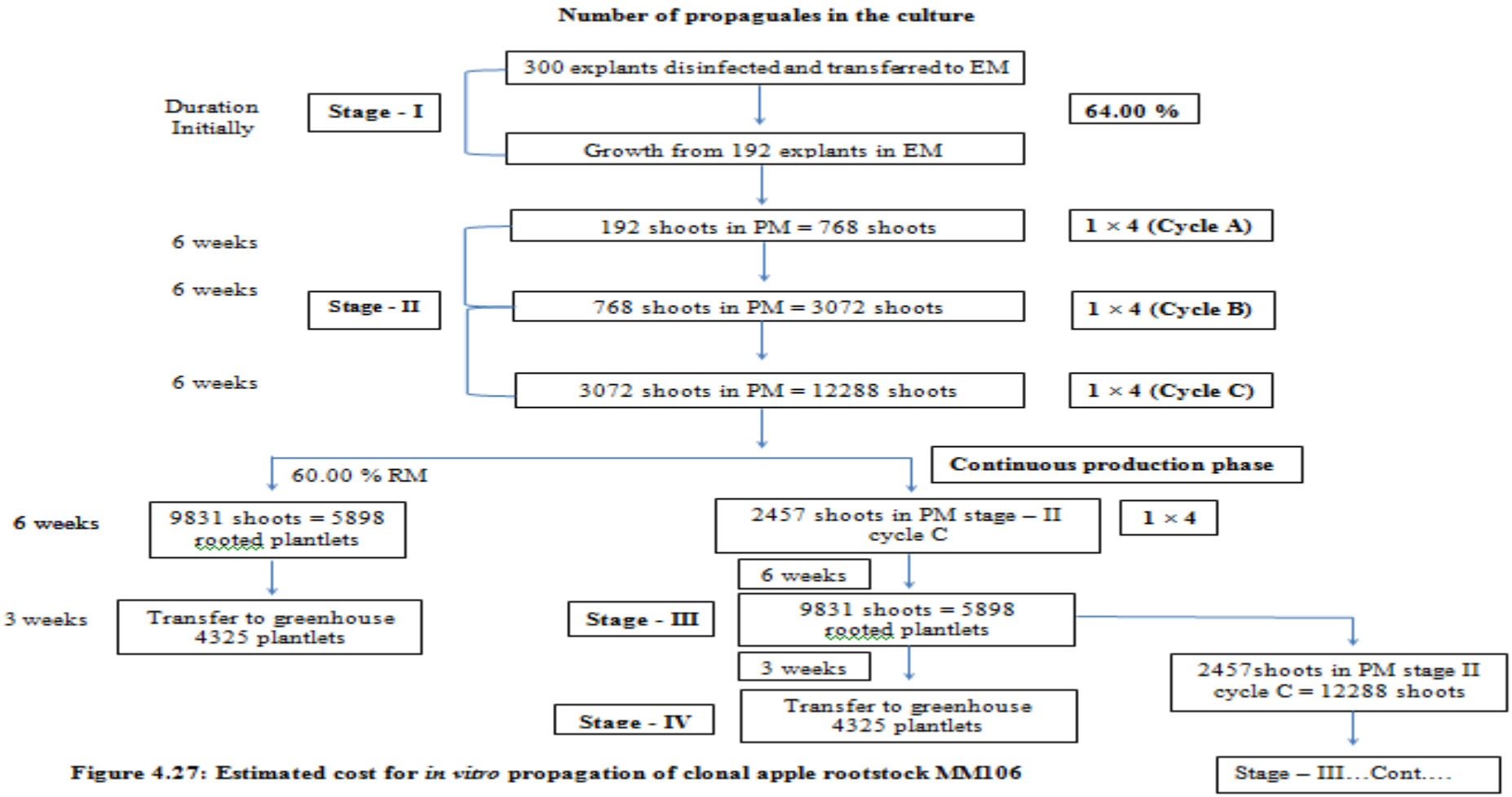


Figure 4.27: Estimated cost for *in vitro* propagation of clonal apple rootstock MM106

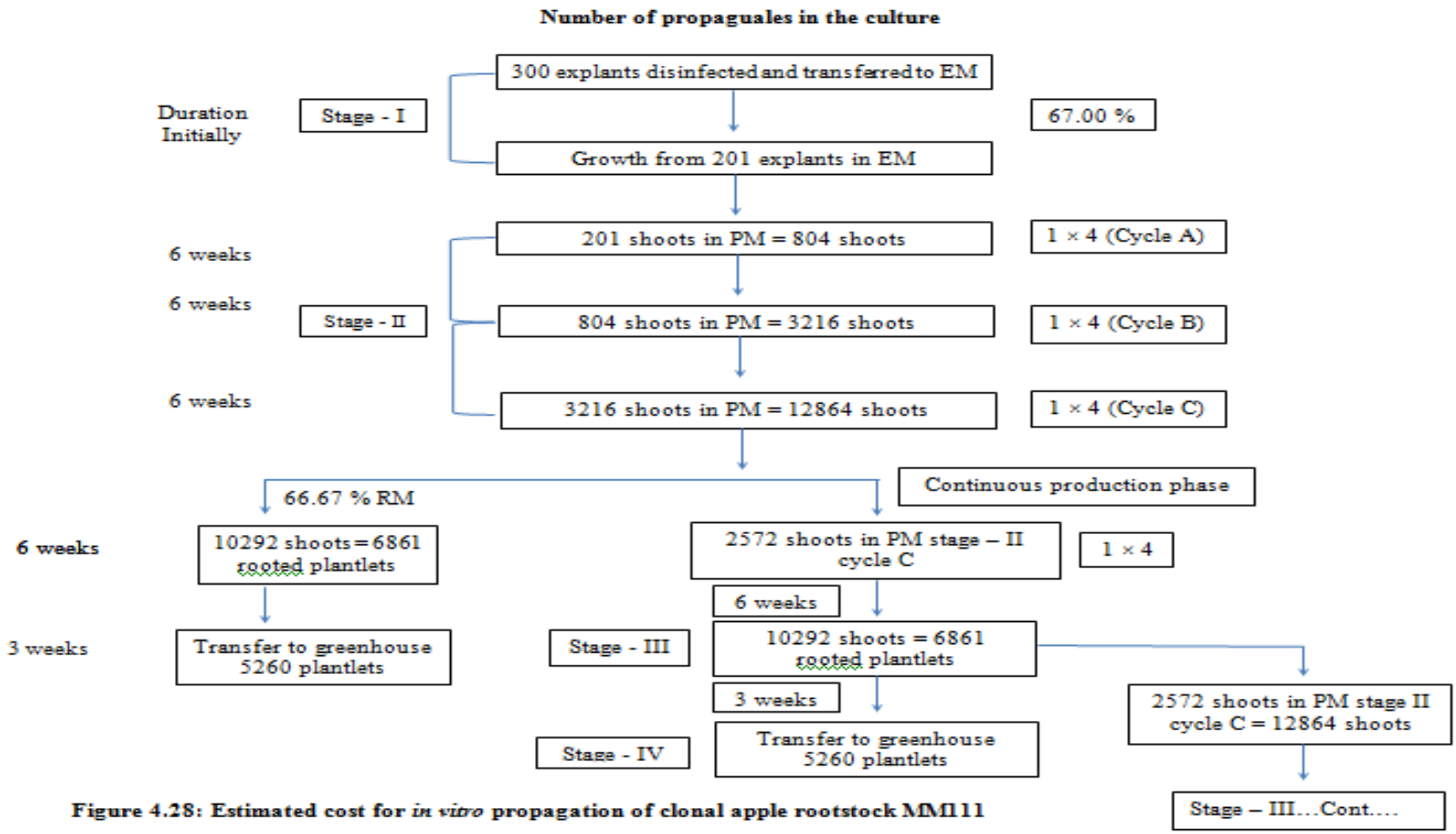


Figure 4.28: Estimated cost for *in vitro* propagation of clonal apple rootstock MM111

Chapter-V

Discussion

CHAPTER - V

DISCUSSION

Micropropagation of woody and semi-woody trees is reported as problematic and to obtain a material without viruses is a long-term and complex process using conventional methods. Many *in vitro* experiments has been conducted for economically important fruit trees to overcome the problems of virus and large-scale planting material production (Amiri and Elahinia, 2011, Mir *et al*., 2010, Sedlak and Paprstein, 2017 and Adıyaman *et al*., 2004). The concept behind micropropagation is based on the phenomenon that explants like shoot tip, nodal segment and leaves when cultured on the medium, can develop large number of shoots identical to their mother plant (true-to-type). In general, response of explant cultured *in vitro* is influenced by several *in vitro* and *in vivo* factors. These include the genotype of the donor plant, nature of the initial inoculum, age of the culture, composition of the culture medium and physiological conditions like temperature, duration and intensity of light and relative humidity prevailing during growth of cultures. Despite the bottlenecks, micro-propagation provides many advantages over conventional propagation methods such as rapid production of plant material in small space, uniformity, *in vitro* storage of germplasm and production of disease and virus free plant material throughout the year. In *in vitro* propagation procedure the organs and tissues are carried through a sequence of steps in which different cultural and environmental conditions are provided. These steps involved in the procedure have been grouped into four different stages *viz.*, culture establishment, shoot proliferation, rooting and acclimatization (Murashige, 1974). For development of a viable micro-propagation procedure/protocol, these four stages are to be standardized individually. Apple being the most important fruit crop of Jammu and Kashmir, contributes towards the major part of the orchardist's annual income. Previously apple plants were raised on seedling rootstocks, but after realizing the potential of clonal rootstocks, most of the orchardists are preferring apple plants on clonal rootstocks. Clonal rootstocks are amenable to rooting so they can be easily micro-propagated under laboratory conditions. But prior to undertaking the commercial micro-propagation of apple clonal rootstocks, the procedure/protocol is to be standardized. Therefore, the investigations entitled "Propagation studies on clonal rootstocks of apple (*Malus* × *domestica* Borkh.)" were carried at Plant Tissue Culture Laboratory

of School of Biotechnology and Tissue Culture Laboratory, Advanced Centre for Horticulture Research (ACHR), Udheyawalla of Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu, Chatha during the year 2018. The results obtained during the course study are discussed in this chapter under the following heads:

5.1 Effect of mercuric chloride and sodium hypochlorite on explant sterilization of apple clonal rootstocks MM106 and MM111

The explants of clonal apple rootstocks MM106 and MM111 collected from open field harbor fungi and bacteria in addition to adhered soil and dirt particles. Thus, it necessitates ~~a~~ thorough and effective surface sterilization of explants before culturing. Debnath (2004) observed that establishment of aseptic cultures is the first critical step in *in vitro* propagation process. Grant and Hemmatt (1999) also reported that surface sterilization of explants is very important step for the successful establishment of explants in cherry and apple. Results obtained in present study revealed that explants treated with mercuric chloride ($HgCl_2$) yielded more satisfactorily results as compared to sodium hypochlorite (NaOCl) with respect to aseptic cultures/un-contaminated cultures and survival of cultures. Percentage of un-contaminated cultures as a result of treatment with $HgCl_2$ (0.05%, 0.1% and 0.2%) and NaOCl (3%, 8% and 12%) for different duration showed that 0.1 per cent $HgCl_2$ for 4 minutes and 8 per cent NaOCl for 20 minutes gave maximum aseptic cultures. The percentage of aseptic cultures in both clonal apple rootstocks (MM106 and MM111) showed decreasing trend after 4 and 20 minutes duration treatment of $HgCl_2$and NaOCl, respectively. However, maximum survival of cultures was obtained with the treatment of 0.1 per cent $HgCl_2$ for 4 minutes and 8 per cent NaOCl for 20 minutes, whereas after 4 and 20 minutes duration of $HgCl_2$ and NaOCl, respectively. The percent survival of cultures decreased during all the months (March, April and May). Between two different explants, nodal segments gave maximum per cent aseptic and survival of cultures during the month of March, April and May. The interaction effect revealed that percentage of aseptic and survival of cultures was recorded maximum with the application of 0.1 per cent $HgCl_2$ for 4 minutes and 8 per cent NaOCl for 20 minutes during whole duration of study. The aseptic culture percentage increased with the duration of exposure upto 4 and 20 minutes treatments

of 0.1 per cent $HgCl_2$ and 8 per cent NaOCl in shoot tip and nodal segment explants, respectively. While lowest aseptic cultures percentage was noticed under control for both the explants. Similarly, the explant survival percentage increased with increasing duration of exposure to 0.1 per cent $HgCl_2$ for 4 minutes and 8 per cent NaOCl for 20 minutes. The lowest survival of cultures was recorded under control for both the explants. The disinfection of an isolated plant material depends on the physiological state of the parent plant at the time of excision. Tissue immaturity is also found to be related with the morphogenetic competence and differentiation in plant tissue which is associated with the loss of regenerative capacity (Sinnott, 1960).

Murashige (1974) reported that besides other factors, the season and type of explant also influence the *in vitro* regeneration success of the explants. The regeneration ability in clonal apple rootstocks is influenced by the month in which it is cultured and highest regenerative ability is found in explants at active growth stage i.e. summer months (Soni *et al*., 2010). In the present study, though 0.1% $HgCl_2$ treatment for 4 minutes provided highest number of aseptic cultures but beyond this, increase in the concentration and duration of exposer of surface sterilants increased the mortality rate and decreased the survival percentage of cultures, which might be due to the phytotoxicity caused by mercuric chloride (Kaur *et al*., 2005 and Kumar *et al*., 2001).

Wolella *et al.* (2017) noted highest explants survival (97.00 %) as well as minimum contamination (3.00 %) when explants disinfected with 2 per cent sodium hypochlorite for 15 minutes and 0.1 per cent mercuric chloride for 7 minutes. Soni *et al.* (2010) reported that axillary buds of Merton-793 apple rootstock when surface sterilized with mercuric chloride for 4 minutes provided highest number of uncontaminated buds, but the survival rate was more in exposure time of 3 minutes. Sakila *et al.* (2007) reported that when nodal segments of strawberry surface sterilized with mercuric chloride for 5 minutes produced maximum percentage of un-contaminated buds. Lizarraga *et al*. (2017) reported that nodal segment from axillary shoots showed highest survival rate at establishment for both apple and pear cultivars. Kaushal *et al.* (2005) and Ramanayake and Yakandwala (1997) suggested that maximum aseptic culture percentage and bud break depends upon degree of contamination and physiological status of the collected explants during various

season of the year. Similar findings has been achieved by Shukla *et al*. (2019), Fatima *et al*. (2018), Padhi and Singh (2017), Yadav *et al*. (2017), Guadie *et al*. (2016), Thakur *et al.* (2016), Arun and Swamy (2015), Mir *et al*. (2013), Zamir and Rab (2014), Ahmed *et al*. (2014), Ghanbari (2014), Jan *et al*. (2013), Vujovic *et al*. (2012), Kaushal *et al*. (2005) and Rana and Singh (2002).

5.2 Effect of antioxidants on per cent culture establishment of apple clonal rootstocks

The results regarding to the response of antioxidants on culture establishment showed a significant influence on the per cent culture establishment of clonal apple rootstocks MM106 and MM111. The clonal rootstock MM106 recorded maximum culture establishment (30.00 %) on MS media containing 10 g polyvinyl pyrrolidone (AM4) which was statistically at par with 28.88 per cent in MS media + 5 g polyvinyl pyrrolidone (AM3). The MS basal medium gave minimum culture establishment (0.00 %). This is might be due to the lack of antioxidants like polyvinyl pyrrolidone, citric acid and ascorbic acid which control the exudation of phenols from the excised portion of the explants in the medium (Kaushal *et al*., 2005). Among two explants used (shoot tip and nodal segment), nodal segment showed higher culture establishment (24.11 %) which was significantly higher culture establishment with the shoot tip (17.11 %). In the interaction effect, media composition AM4 showed maximum culture establishment (32.22 %) for nodal segment followed by AM3 (31.11 %), while shoot tip showed 27.77 per cent culture establishment with AM4 followed by AM3 (26.66 %). However, for both explants, minimum culture establishment was recorded as 0.00 per cent under control.

In clonal apple rootstock MM111, maximum culture establishment (41.61 %) was recorded under AM4 which was statistically at par with AM3 (40.61 %). However, minimum culture establishment was recorded as 0.00 per cent under control. Between the two explants, nodal segment explants gave maximum culture establishment of 34.59 per cent which was significantly higher than the culture establishment with shoot tip (26.48%). The interaction data revealed that nodal segment with AM4 gave maximum culture establishment of 47.88 per cent followed by 46.66 per cent in AM3, while shoot tip recorded 35.33 per cent culture

establishment followed by 34.55 per cent with AM3. The MS basal medium recorded minimum culture establishment of 0.00 per cent for both the explants.

The phenol exudation was the major problem during culture establishment, which increases the rate of oxidative browning that causes death of the majority of cultured explants (Nishchal *et al*., 2018). Volz and McGhie (2011) reported that all *Malus* species contain high level of phenols that inhibits the extent of culture establishment. In the present study, among different antioxidants, polyvinyl pyrrolidone was found to be more effective antioxidant to minimize the phenol exudation from the excised portion of the explants, while citric acid and ascorbic acid revealed comparatively lower effect on phenol exudation as compared to polyvinyl pyrrolidone. The effect of various antioxidants in controlling browning and phenolic excretion varies between plants and species (Jakhar *et al*., 2019). This could be due to the specificity of different antioxidants like polyvinyl pyrrolidone, citric acid, ascorbic acid and activated charcoal to certain plants and species (Prajapati *et al*., 2003) who found that culture browning can be successfully controlled by the addition of polyvinyl pyrrolidone (PVP) into the medium.

Regarding the season of taking explants, our results corroborate with the results of Modgil *et al*. (1999) who observed that explants of Tydeman's Early Worcester apple plants showed maximum sprouting (75.00 %) during spring and summer months in comparison with those collected during other seasons (30.00 %). Similarly, Webster and Jones (1991) and Hutchinson (1984) agreed that the rate of browning and phenol exudation of apple explants decreases considerably when they are collected in the summer months. Our results are also in accordance with the results obtained by Singh and Patel (2016), Singh and Khawale (2006), Tang and Newton (2004) Murkute *et al*. (2004), Singh *et al*. (2002) and Pandeliev *et al*. (1990).

5.3 Effect of media composition on per cent culture establishment of clonal apple rootstocks

Among different media, MS media + 1.0 mg/l BAP + 1.0 mg/l GA_3 (EM8) gave highest culture establishment of 52.77 per cent in clonal apple rootstock MM106 which was statistically at par with 51.66 per cent obtained in MS + 1.0 mg/l BAP (EM2). The MS basal medium (without BAP and GA_3) showed minimum culture

establishment (0.00 %). The reason for no shoot regeneration on this medium might be due to the lack of optimum plant growth regulators like cytokinin which is required for the adventitious shoot formation (Lizarraga *et al*., 2017). Between the two explants (shoot tip and nodal segment), nodal segment explants gave higher culture establishment (27.57 %) which was significantly higher than the culture establishment with shoot tip (23.96 %). In the interaction effect, nodal segment under EM8 gave maximum culture establishment (57.77 %) followed by EM2 (23.96 %), while shoot tip gave maximum culture establishment (47.77 %) followed by EM2 (46.66 %). However, minimum culture establishment (0.00 %) for both the explants was recorded under control.

In apple rootstock MM111, maximum culture establishment of 68.89 per cent was recorded under EM8 (MS + 1.0 mg/l BAP + 1.0 mg/l GA_3) which was statistically at par 67.78 per cent recorded in EM2 (MS + 1.0 mg/l BAP). However, minimum culture establishment (0.00 %) was recorded under control. The reason for no shoot formation on control might be due to the lack of optimum concentration of plant growth regulators which are required for the shoot formation from the cultured explants (Guadie *et al*., 2016). Between the two explants, nodal segment explants gave maximum culture establishment (41.84 %) which was significantly higher than the shoot tip explants (30.83 %). The interaction data revealed that nodal segment under EM8 gave maximum culture establishment of 76.66 per cent followed by 75.56 per cent under EM2, while shoot tip recorded 61.11 per cent culture establishment followed by 60.00 per cent in EM2. The MS basal medium recorded minimum culture establishment (0.00 %) for both the explants.

The minimum number of days taken for culture establishment (29.32 days) in clonal apple rootstock MM106 was recorded under EM8 (MS + 1.0 mg/l BAP + 1.0 mg/l GA_3) which was statistically at par with 29.87 days in EM2 (MS + 1.0 mg/l BAP) and was followed by 30.52 days in EM11 (MS + 2.0 mg/l BAP + 1.0 mg/l GA_3). The maximum number of days (38.32 days) taken for culture establishment was recorded under EM16 (MS + 3.0 mg/l BAP + 3.0 mg/l GA_3). Between the two explants (shoot tip and nodal segment), nodal segment explants took minimum number of days (30.52) for culture establishment which was significantly higher than the shoot tip explant (36.46 days). In the interaction effect, nodal segment explants

under EM8 took minimum number of days (26.33 days) for culture establishment followed by 26.47 days in EM2, while shoot tip took 32.20 days followed by 33.27 days in EM2. However, maximum number of days taken for culture establishment were recorded as 35.33 days and 41.30 days in nodal segment and shoot tip explants, respectively in EM16. In clonal apple rootstock MM111, minimum number of days (28.80 days) taken for culture establishment was also recorded under EM8 which was statistically at par with 28.87 days in EM2 and was followed by 30.43 days in EM8. However, maximum number of days (38.32 days) taken for culture establishment was recorded in EM16. Between the two explants, minimum number of days (29.98) taken for culture establishment was recorded by nodal segment cultured on EM8 which was significantly higher than the shoot tip (35.92 days). The interaction effect indicated that nodal segment explants took minimum number of days for culture establishment (25.40) on EM8 followed by 25.47 days in EM2, while EM8 took minimum number of days (32.20) for culture establishment followed by EM2 (32.27 days) for shoot tip. The maximum number of days taken for culture establishment in nodal segment and shoot tip were 34.13 days and 40.27 days, respectively recorded on EM16. Arello *et al*. (1991) also reported that medium supplemented with BAP and GA_3 alone or in combination was more effective for meristem and nodal segment establishment.

According to Skoog and Miller (1957) quantitative interaction between diverse growth factor may have decisive role in organogenesis of cultures. In the present study, higher concentration of BAP and GA_3 alone or in combination decreased the rate of culture establishment percentage in clonal apple rootstocks MM106 and MM111. This may be due to the genotypic difference among clonal apple rootstocks or due to the endogenous level of hormones, nutrients, metabolites and interaction between various factors. Further, Singh *et al*. (2004) opined that depending upon the endogenous factors, requirement of exogenous applications vary to get optimum response of cultured explants. Lizzarraga *et al*. (2017) reported highest survival rate of 55.0 per cent with combined application of BAP and GA_3 during establishment stage of apple cultivar Principe Grande. However, Rodriguez *et al*. (1991) reported that presence of GA_3 in the medium inhibits the culture establishment. Guadie *et al*. (2016) reported that after 30 days of growth on culture

initiation medium a maximum of 96.7 per cent (29 out of 30) MM106 shoot explants were initiated on MS medium containing 1.0 mg/l BAP in combination with 0.1 mg/l IBA. Boudabous *et al.* (2010) reported that MS medium augmented with BAP (1.0 mg/l) along with auxin resulted in maximum sprouting (85.0 %). Soni *et al.* (2010) noted that out of different combinations, media containing 0.5 mg/l - 1.0 mg/l BAP were found to be more suitable for establishment of apple explants. Dalal *et al*. (2006) reported that 1.0 mg/l BAP combined with 0.1 mg/l IBA is best for *in vitro* culture initiation of clonal apple rootstock M9. Kaushal *et al*. (2005) reported that MS medium supplemented with BAP (1.0 mg/l) and GA_3 (0.5 mg/l) resulted in significant increase in culture establishment percentage of clonal apple rootstock MM111. Mir *et al*. (2013) also noticed that MS medium supplemented with BAP and GA_3 resulted in maximum culture establishment percentage in clonal apple rootstock MM106. Findings of present experiment are also in conformity with the results of Chakrabarty *et al*. (2003), Sharma *et al*. (2000),Yepes and Aldwinckle (1994) and Hirabayashi *et al*. (1987).

5.5 Effect of media composition on proliferation of explants of clonal apple rootstock

In clonal apple rootstock MM106, MS media supplemented with 1.0 mg/l BAP and 1.0 mg/l GA_3 (MM8) took minimum number of days (49.33) for shoot proliferation which was statistically at par with 49.78 days in MS + 1.0 mg/l BAP (MM2). The highest number of days (58.30) taken for shoot proliferation was recorded on MS + 3.0 mg/l BAP + 3.0 mg/l GA_3 (EM16). Between two explants, nodal segment explants on MM8 took minimum number of days (51.37) for shoot proliferation which was significantly higher than the shoot tip (57.81 days). The interaction data revealed that explants on MM8 took minimum number of days (46.26) for shoot proliferation in nodal segment explants followed by 46.56 days in MM2, while in shoot tip explant, minimum number of days (52.40) taken for shoot proliferation was recorded on MM8 followed by MM2 (53.00 days). However, maximum number of days taken for shoot proliferation in nodal segment and shoot tip were 55.67 and 61.47 days, respectively on MM16. In clonal apple rootstock MM111, explants took minimum number of days (47.77) on MM8 for shoot proliferation which was statistically at par with 48.22 days on MM2. Between the two

explants, nodal segment explants under MM8 took minimum number of days (41.81) for shoot proliferation which was statistically higher than that through shoot tip (56.27 days). The interaction data revealed that nodal segment explants under MM8 took minimum number of days (44.70) for shoot proliferation followed by 45.00 days in MM2, while in shoot tip, minimum number of days (50.84) for shoot proliferation was recorded under MM8 followed by 51.44 days in MM2. However, maximum number of days taken for shoot proliferation in nodal segment and shoot tip were 54.11and 60.15 days respectively in MM16.

The data with respect to number of shoots per explant of clonal apple rootstocks revealed that in clonal apple rootstock MM106, media MM8 (MS + 1.0 mg/l BAP and 1.0 mg/l GA_3) recorded maximum number of shoots per explant (3.58) which was found statistically at par with 3.48 shoots in MM2 (MS + 1.0 mg/l BAP), while minimum number of shoots per explants (0.67) were recorded in media MM16 (MS + 3.0 mg/l BAP + 3.0 mg/l GA_3). Between the two explants, nodal segment explants recorded maximum number of shoots per explants (2.83) which was significantly superior to 1.26 shoots per explants observed in shoot tip. In the interaction effect, maximum number of shoots per explants (4.76) was recorded in nodal segment with MM8 followed by 4.66 in MM2, while shoot tip recorded 2.40 shoots per explant with MM8 followed by 2.30 in MM2. However, minimum number of shoots per explant for shoot tip and nodal segment were recorded as 0.33 and 1.00, respectively on MM16. The clonal apple rootstock MM111 recorded maximum number of shoots (3.93) on MM8 which was statistically at par with 3.83 on MM2. The minimum number of shoots (1.00) per explant was recorded on MM16. Between the two explants, nodal segment explants recorded maximum number of shoots (3.49) per explant which was significantly superior to shoot tip (1.37). In the interaction effect, maximum number of shoots (5.43) per explant was recorded by nodal segment on MM8 followed by 5.33 on MM2, while 2.43 shoots per explants were recorded through shoot tip on MM8 followed by 2.33 shoots on MM2. However, minimum number of shoots (0.33 and 1.66) per explant for shoot tip and nodal segment, respectively was recorded under MM16.

The data with respect to length of the longest shoot showed that different media compositions had a significant influence on length of the longest shoot of

clonal apple rootstocks MM106 and MM111. In clonal apple rootstock MM106, maximum shoot length was recorded as 3.31 cm on MM8 (MS + 1.0 mg/l BAP and 1.0 mg/l GA_3) which was significantly superior over rest of the media combinations. Between the two explants, nodal segment recorded maximum shoot length (2.73 cm) which was significantly higher than the shoot length (1.77 cm) through shoot tip. In the interaction effect, nodal segment explants recorded maximum shoot length (3.42 cm) under MM8, while shoot tip recorded 3.19 cm shoot length. However, minimum shoot length was recorded as 1.77 cm and 2.10 cm for shoot tip and nodal segment, respectively on MM16 (MS + 3.0 mg/l BAP + 3.0 mg/l GA_3). In clonal apple rootstock MM111, MM8 recorded maximum shoot length as 3.34 cm which was statistically superior over rest of the media combinations. Between the two explants, nodal segment explants recorded higher shoot length (2.77 cm) which was significantly superior to shoots length (2.49 cm) obtained through shoot tip. The interaction study revealed that nodal segment explants recorded maximum shoot length (3.47 cm) on MM8 followed by 3.30 cm on MM2, while shoot tip recorded 3.21 cm shoot length on MM8 followed by 3.11 cm on MM2. However, minimum shoot length was recorded as 1.82 cm and 2.15 cm in shoot tip and nodal segment explants, respectively on MM16.

The maximum number of leaves (10.00) in clonal apple rootstock MM106 was recorded under MM8 (MS + 1.0 mg/l BAP and 1.0 mg/l GA_3) which was statistically at par with 9.66 in MM2 (MS + 1.0 mg/l BAP). Between the two explants, nodal segment recorded higher number of leaves (7.27) which was significantly superior to the number of leaves (5.89) through shoot tip explant. The interaction data revealed that maximum number of leaves 10.33 and 9.67 in nodal segment and shoot tip explants, respectively was recorded with MM8. However, minimum number of leaves was recorded as 3.00 and 4.00 in shoot tip and nodal segment explants, respectively on MM16. The maximum number of leaves (10.66) in clonal apple rootstock MM111 was recorded with MM8 which was statistically at par with MM2 (10.34 leaves). Between the two explants, nodal segment recorded maximum number of leaves (7.93) which was significantly superior 6.51 leaves obtained in shoot tip explant. The interaction data revealed that maximum number of leaves 11.00 and 10.33 in nodal segment and shoot tip explants, respectively was

recorded with MM8. However, minimum number of leaves was recorded as 3.33 and 4.67 in shoot tip and nodal segment explants, respectively with MM16.

In the present investigation, MS medium supplemented with BAP (1.0 mg/l) and GA_3 (1.0 mg/l) along with 0.2% NAA was found to be more effective for shoot proliferation as compared to the higher concentration of BAP and GA_3 alone or in combination. This may be due to the action of phytohormones (Kour and Singh 2012). Ahmed *et al*. (2014) suggested that hormone treatment starts the cell on a specific development pathway and the alternative view is that hormone responsive cells are already determined and hormones evoke the expression of the combined state. Hence, the observed differences in the requirement of growth hormones for the shoot proliferation of explants as reported by different workers as well as in the present investigation could be attributed to the differential requirement of the growth hormones. Shoot tips as well as nodal segments in active growth phase, whether distal or basal are more favorable source of explants (Arello *et al*., 1991). Shoot proliferation of apple has been found to be influenced by hormone level in lower concentration, genotype and their interaction (Dobranszki and Silva, 2010). The critical concentration of cytokinin and auxin has been reported to be very important for regeneration of explants in the medium (Goyal and Bhadauria, 2007 and Silva *et al*., 2006). Ohyama and Oka (1987) also reported the inhibitory effect of BAP on shoot proliferation of mulberry at concentrations higher than 1.0 mg/l. Some of the reports also confirmed that endogenous levels of hormones of apple tissue makes them unable to stimulate on their own the sprouting of axillary buds as well as multiple shoot formation (Andreu and Marin, 2005; Mereti *et al.* 2002, Carelli and Echeverrigaray 2002, Mhatre *et al.* 2000 and Shibli *et al.* (1997).

Meneguzzi *et al*. (2017) developed a suitable protocol for micropropagation of apple rootstock G814 and reported that BAP (1.0 mg/l) showed best results at multiplication phase and produced maximum number of shoots per explant. MS medium supplemented with BAP and GA_3 has also been found to significantly increase in number of shoots, shoot length and number of leaves per explants of CG41 apple rootstock (Castillo *et al.* (2015). MS medium in combination with BAP also favors maximum proliferation rate with an average of 5.45 shoots per explants, 2.89 shoots per explant in Azayesh-Esfahan apple rootstock (Ghanbari, 2014).

Tabalvandani *et al.* (2014) also reported that 1.0 mg/l BAP along with NAA promote highest proliferation rate (6.73), number of shoots (3.00) in Irani apple rootstoock AZ × M9. Mir *et al*. (2013) noticed MS medium supplemented with 1.0 mg/l BAP and 0.5 mg/l GA_3 resulted in higher number of shoots, length of the shoots and number of leaves per shoot in clonal apple rootstock MM106.

Arab *et al.* (2014) reported that MS medium supplemented with 1.0 mg/l BAP resulted in 8.5 shoots per explant and 1.51 cm long shoot. Mir *et al*. (2013) reported maximum number of shoots (13.00), length of shoots (9.67 cm), leaf length (3.47 cm) and leaf number (15.3) on MS media supplemented with 1.0 mg/l BAP and 0.5 mg/l GA_3. Moreover, increasing the concentration of BAP upto 3.0 mg/l, shoot number did not significantly enhanced resulting in elongated shoots with short length leaves. Amiri and Elahinia (2011) also reported that lower concentration of plant growth regulators, particularly 6-benzyladenine and GA_3 significantly affect the shoot induction and produced more number of shoots and leaves per explants in three apple rootstocks (M9, M27 and MM106).

Soni *et al.* (2010) reported higher concentration of BAP along with GA_3 may increase the shoot number but decrease the shoot length, whereas the lower concentration led to the longer shoots. MS medium containing 1.0 mg/l BAP was found to be adequate for the shoot multiplication in apple explants (Butiuc-Keul *et al.*, 2010). Boudabous *et al.* (2010) observed that multiple shoots were induced with varying frequencies (9.2 ± 90%) by culturing shoot tips of Douce de Djerba apple plants on medium supplemented with 0.5 to 4.0 mg/l BAP but as the concentration of BAP exceeded to 4.0 mg/l, there was no enhancement in multiple shoot induction. They also reported that MS medium in combination with 1.0 mg/l BAP along with auxin resulted in maximum number of leaves (10.0) and maximum shoot length (3.8 cm). Kaushal *et al*. (2005) reported that maximum shoot proliferation rate (1: 5) and maximum shoot length (1-4 cm) were recorded with MS medium supplemented with 1.0 mg/l BAP along with 0.5 mg/l -1.0 mg/l GA_3. They also reported that BAP was found to be most effective cytokinin for axillary buds and shoot apices but the response was dependent upon the concentration and combination used.

Hartman *et al*. (2004) suggested that auxins should be either completely absent, or used in small concentration in shoot multiplication medium. Santa-Catarina

et al. (2001) concluded that for clonal apple rootstock Selection 69, BAP along with GA_3 at lower concentration produced greatest number of shoots. Modgil *et al*. (1999) reported maximum axillary shoots produced from nodal segments of Tydeman's Early Worcestor apple plants as compared to other explants. Higher concentration of plant growth regulators particularly GA_3 in the medium inhibits shoot proliferation and increases the number of days required for shoot proliferation (Ellot, 1972). Although, the presence of GA_3 at lower concentration in the medium enhances length of the shoots as it is responsible for the phenomenon apical dominance (Litwinczuk *et al*., 2009). These results indicate that there is not exact concentration of cytokinin and for every selection, even from same breeding programme, the concentration must be analyzed. Further, present findings are also in conformity with the results of Lizarraga *et al*. (2017), Wang *et al*. (2016), Aygun and Dumanoglu (2015), Mert and Soylu (2010), Haripyaree *et al*. (2011), Butic-Keul *et al*. (2010), Dalal *et al*. (2006), Silva *et al*. (2006), Modgil *et al*. (2005), Miri *et al*. (2003), Zhang *et al*. (2004), Hossain *et al*. (2001), Sharma *et al*. (2000), Pua *et al*. (1983) and Lundergan and Janick (1980).

5.6 Effect of media composition on *in vitro* rooting of clonal apple rootstocks

Media combination RM6 (Half MS + 1.0 mg/l NAA) recorded rooting in 63.34 per cent cultures, which was significantly superior over rest of the media combinations, while minimum cultures rooted (0.00 %) was recorded on half MS basal media (control). Between the two different clonal apple rootstocks, higher rooting in (34.58 %) cultures was obtained in MM111 which was significantly higher than 28.54 per cent rooting in MM106. The interaction data revealed that maximum cultures rooted (66.67 %) on RM6 in MM111 followed by 60.00 per cent in MM106. However, minimum rooting in cultures (0.00 %) was recorded under control for both the clonal rootstocks. Results pertaining to the effect of media compositions on number of days taken for root initiation of clonal apple rootstocks revealed that minimum number of days (13.50) taken for root initiation was recorded on RM6 which was significantly superior to all other media combinations. Between the two clonal apple rootstocks, MM111 took minimum days (16.73) for root initiation which was significantly higher than 17.53 days in MM106. In the interaction effect, media combination RM6 took minimum numbers of days (13.00) for root initiation in clonal apple rootstock MM111 followed by 14.00 days in MM106. The significant variation

for number of roots per explant of clonal apple rootstocks MM106 and MM111was also noticed. Media combination RM6 recorded maximum number of roots per explant (5.34) which was significantly higher over rest of the media combinations. On an average, maximum number of roots (3.33) was observed in MM111 which was significantly higher than 2.71 roots in MM106 rootstock. In the interaction effect, RM6 recorded maximum number of roots per explant (5.67) in clonal apple rootstock MM111 followed by 5.00 roots per explants in MM106. However, minimum number of roots per explant was recorded as 1.67 and 1.00 on RM16 for MM111 and MM106, respectively. The results for the effect of different media combinations on length of the longest root of clonal apple rootstocks revealed that different media compositions had a significant effect on length of the longest root. The maximum root length (3.73 cm) was recorded on RM6 which was significantly higher than all other media combinations and was followed by 3.64 cm in RM3, 3.34 cm in RM9 and 3.24 cm in RM12. Between two different apple clonal rootstocks, MM111 showed higher root length (2.65 cm) which was significantly superior to 2.57 cm root length in MM106. In the interaction effect, maximum root length was recorded as 3.75 cm and 3.70 cm on RM6 for clonal apple rootstock MM111 and MM106, respectively. However, minimum root length was recorded as 1.80 cm and 1.69 cm on RM16 for MM111 and MM106, respectively.

In the present investigation, half strength MS media supplemented with 1.0 mg/l NAA along with 0.2 per cent activated charcoal was found to be superior for *in vitro* rooting of clonal apple rootstocks. It is well known that half strength MS medium reduces the callus formation (Dalal *et al*., 2006). Some researchers proposed the superiority of half strength MS medium for root induction (Choudhary *et al*., 2015, Alam and Barua, 2015, Zou, 2010, Hartmann *et al*., 2004 and Hossain, 2003). They reported that the use of activated charcoal in the root induction medium enhances the root proliferation and reduces the callus growth. Addition of activated charcoal to the rooting medium favors more effectual rooting in clonal apple rootstocks, as it helps in absorption of auxins present in the medium (Sharma *et al*., 2007, Mahajan *et al*., 2001, Kour *et al*., 2005 and Gautam *et al*., 2001). Renu *et al*. (2018) reported that out of the different auxins tested for *in vitro* rooting in Golden Delicious apple explants, NAA supported highest rooting with average of 7.66

numbers and root length of 2.2 cm. Kour and Singh (2012) obtained maximum rooting percentage (73.33 %) in minimum number of days (19.29) in rough lemon with half strength MS medium supplemented with 1.0 mg/l NAA. Soni *et al*. (2010) during *in vitro* multiplication of Merton 793 apple rootstock reported that presence of NAA in the medium was found to be more efficient than IBA and IAA with respect to rooting percentage (55.20 %). Negi (2011) reported that maximum rooting percentage (66.78 %) with maximum root number (8.00) and length (3.80 cm) were observed with MS medium containing NAA for clonal apple rootstock Merton-793. However, Soni (2005) reported highest rooting frequency and good root quality in two step rooting, using IBA. This might be due to the difference in the plant growth regulators and their concentrations used in the multiplication medium. Dihiz *et al*. (2007) reported consistently more number of roots produced with MS medium supplemented with the moderate concentration of NAA, IBA and activated charcoal. Magyar *et al*. (2002) studied the effect of activated charcoal and NAA on root elongation of *in vitro* apple cultivar Royal Gala and observed that the presence of activated charcoal in the medium enhanced root elongation and root number due to the better adsorption of NAA. Our results are in conformity with the results of Modgil *et al*. (2017), Atawia *et al*. (2016), Hu (2015), Shabani *et al*. (2015), Kour and Singh (2012), Mohamed (2012), Haripyaree (2011), Saini *et al*. (2010), Thorpe (2008), Demiral and Ulger (2008), Dihiz *et al*. (2007), Begum *et al*. (2003), Al-Bahrany (2002), Al-Khayri and Al-Bahrany (2001), Das *et al*. (2000), Arello *et al.* (1991) and Lane and McDougald (1982).

5.7 Effect of soil medium on survival of *in vitro* raised plantlets of clonal apple rootstocks

The effect of different soil medium on per cent survival of *in vitro* raised plantlets of clonal apple rootstocks MM106 and MM111 showed a significant variation among different medium combinations. Media combination soil + sand + vermiculite + FYM (SM7) recorded maximum survival (75.00 %) which was significantly higher than all other soil mediums and was followed by 60.00 per cent with soil + sand + vermiculite (SM5) and 50.00 per cent with soil + sand + FYM (SM6), while minimum survival (0.00 %) was recorded under 100 per cent sand (SM1). Between two rootstocks, MM11 recorded maximum survival (43.33 %) which

was significantly higher than 37.14 per cent for MM106. The interaction data revealed that maximum survival (76.67 %) was recorded on SM7 in clonal apple rootstock MM111 followed by 73.33 per cent for MM106 on SM7. Minimum survival (0.00 %) was recorded with sand 100 per cent for both the rootstocks. The data with respect to the effect of different soil medium on plant height showed that maximum plant height (3.26 cm) was recorded under SM7 which was significantly superior over rest of the soil media and was followed by 2.80 cm on SM5 and 2.03 cm on SM6, whereas the minimum plant height (0.56 cm) was recorded on SM2. Between two clonal rootstocks, MM111 recorded maximum plant height (1.74 cm) which was significantly superior to 1.64 cm for MM106. In the interaction effect, MM111 rootstock recorded maximum plant height (3.30 cm) on SM7 followed by 3.23 cm for MM106 on media SM7. However, for both the rootstocks MM111 and MM106, minimum plant height was recorded as 0.60 cm and 0.52 cm, respectively on 100 per cent soil (SM2). Maximum number of leaves (9.50) was obtained on SM7 which was significantly higher than all other soil media and was followed by 7.50 on SM5 and 6.50 on SM6, while minimum number of leaves (4.50) were obtained on SM2. Between the two clonal apple rootstocks, MM111 recorded maximum number of leaves (7.00) which was significantly higher than 6.11 leaves on MM106. In the interaction effect, rootstock MM111 recorded maximum number of leaves (10.00) on SM7 followed by 6.11 leaves on SM7 for MM106. However, minimum number of leaves were recorded as 5.00 and 4.00 in clonal apple rootstocks MM111 and MM106, respectively on SM2.

In vitro rooting stage is followed by hardening which involved the transfer of plants from *in vitro* conditions to soil to impart tolerance to moisture stress, for conferring a degree of resistance against certain pathogens and conversion of the plant from heterotrophic to autotrophic stage (Hazarika, 2003 and Murashige, 1974). Hardening is done so as to make *in vitro* raised plantlets to adapt them to the natural environment which is a critical process because of their anatomical and physiological peculiarities. Acclimatization is essential in the case of *in vitro* produced plants because *in vitro* plant material is not adopted to the *in vivo* conditions (Brainerd and Fuchigami, 1981). The success in acclimatization of *in vitro* produced plants is largely dependent on the post transfer growth conditions (Ziv, 1986). The continuous

light availability with air-conditioned room as well as constant and low temperature may have a favorable effect on morphological and physiological adoption for developing typical terrestrial water content mechanism. The method of covering *in vitro* raised plantlets followed by misting in greenhouses/ playhouses for initial period and subsequently removing the cover is a gradual process, which was successfully adopted by a number of workers for hardening the plantlets (Kour *et al*., 2005, Karwa, 2003 and Murashige, 1974).

Among various soil mediums tried, the mixture containing sand + soil + vermiculite + FYM (1:1:1:1, v/v/v/v) was found to be superior. Physical, chemical and biological properties of the soil medium are also important for the successful establishment of *in vitro* produced plantlets. Better performance media containing FYM may be attributed to its ability to improve biological properties of the soil. On the other hand, sand may be responsible for providing sufficient aeration and vermiculite has high water holding capacity. Hence, mixing sand, soil, vermiculite and FYM in equal volumes might have helped in giving better grip for roots ample aeration and sufficient amount of organic matter.

Similar results have been obtained in different crops by Lalrinsanga *et al*. (2013), Kour and Singh (2012), Bhatt *et al*. (2012), Soni *et al*. (2010), Zou (2010), Dobranszki and Silva (2010), Kaur *et al*. (2005), Kaur and Chopra (2004), Singh *et al*. (2002) and Babylatha (1993).

5.7 Economics of *in vitro* raised plantlets of clonal apple rootstocks

It is clear from the results that commercial *in vitro* production of clonal apple rootstocks *viz.*, MM106 and MM111 is economically feasible. On the basis of results obtained during investigation, 4325 MM106 plantlets can be produced every month with an estimated cost of ₹ 44.56 per plant which includes ₹ 41.38 per plantlets during tissue culture production phase and `3.18 per plantlet during greenhouse production phase. Whereas, for MM111 apple rootstock, 5260 plantlets can be produced every month with an estimated cost of ₹ 36.95 per plant which includes ₹ 32.29 per plantlets during tissue culture production phase and ₹ 2.66 per plantlet during greenhouse production phase. Since multiplication is the major economic criterion for successful commercial tissue culture propagation, the proliferation rate

of stage II determines the feasibility of *in vitro* propagation of a given species (Kour *et al.* 2009 and Bhojwani and Razdan, 1983). It is also evident from the results that the estimated production cost per plantlets can further be reduced by increasing the multiplication rate and by employing the labor saving alternatives.

Chapter-VI

Summary and Conclusion

CHAPTER –VI

SUMMARY AND CONCLUSION

The present investigation "Propagation studies on clonal rootstocks of apple (*Malus* × *domestica* Borkh.)" was conducted at Plant Tissue Culture Laboratory of School of Biotechnology and Tissue Culture Laboratory, Advanced Centre for Horticulture Research (ACHR), Udheywalla of Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu, Chatha during the year 2018. The results obtained from the investigation are summarized and concluded as follows:

6.1 Effect of surface sterilization

Treatment of 0.1% $HgCl_2$ for 4 minutes was found to be optimum for surface sterilization of explants, which resulted in maximum percentage of aseptic and survival of cultures.

6.2 Effect of antioxidants on percent culture establishment

The different antioxidants *viz.*, polyvinyl pyrrolidone, citric acid and ascorbic acid showed a significant effect on per cent culture establishment of clonal apple rootstocks MM106 and MM111. Media combination MS + 10 g PVP (EM4) recorded maximum culture establishment, while minimum culture establishment was recorded under control.

6.3 Effect of media composition on per cent culture establishment

Media combination MS + 1.0 mg/l BAP + 1.0 mg/l GA_3 (EM8) yielded maximum culture establishment of 68.89 per cent and 52.77 per cent in clonal apple rootstock MM111 and MM106, respectively. However, minimum culture establishment (0.00%) was recorded under control.

6.4 Effect of media composition on number of days taken for culture establishment

The minimum number of days taken for culture establishment were 28.80 days and 29.32 days in clonal apple rootstock MM111and MM106, respectively on media combination MS + 1.0 mg/l BAP + 1.0 mg/l GA_3 (EM8).

6.5 Effect of media composition on shoot proliferation

The different media combinations showed significant effect on shoot proliferation of clonal apple rootstocks. Media MS + 1.0 mg/l BAP + 1.0 mg/l GA_3 (MM8) took minimum number of days as 47.77 and 49.33 days for shoot proliferation in clonal apple rootstock MM111 and MM106, respectively.

Highest number of shoots per explants recorded as 3.93 and 3.58, shoot length as 3.34 cm and 3.31 cm and number of leaves as 10.66 and 10.00 in clonal apple rootstock MM111 and MM106, respectively on media combination MS + 1.0 mg/l BAP + 1.0 mg/l GA_3 (MM8).

6.6 Effect of media composition on *in vitro* rooting

Different media compositions had a significant influence on *in vitro* rooting of clonal apple rootstocks. Media combination Half MS + 1.0 mg/l NAA (RM6) recorded 66.67 per cent and 60.00 per cent rooted cultures in clonal apple rootstocks MM111 and MM106, respectively. However, minimum rooted cultures (0.00%) were recorded under control.

The minimum number of days taken for root initiation was 13.00 and 14.00 observed in clonal apple rootstocks MM111 and MM106, respectively on media combination RM6. Different media combinations also showed a significant effect on number of roots and number of leaves per explant of clonal apple rootstocks. RM6 recorded maximum number of roots per explant as 5.67 and 5.00 and root length as 3.75 cm and 3.70 cm in clonal apple rootstock MM111 and MM106, respectively.

6.7 Effect of soil medium on *in vitro* raised plantlets

Different soil medium showed a significant effect on percent survivality, plant height and number of leaves per plantlets. The maximum survival was recorded as 76.67 per cent and 73.33 per cent in clonal apple rootstocks MM111 and MM106, respectively on media combination soil + sand + vermiculite + FYM (SM7). Maximum plant height was 3.30 cm and 3.23 cm and maximum number of leaves as 10.00 and 9.00 recorded in clonal apple rootstocks MM111 and MM106, respectively on media (SM7).

6.8 Economics of *in vitro* raised plantlets

The cost of producing one *in vitro* grown clonal apple rootstock of MM106 and MM111 including 6 weeks hardening was found to be ₹ 44.56 and ₹ 36.95, respectively.

Conclusion

The protocol developed in current investigation for the mass multiplication of virus and disease free plantlets from the shoot tip and nodal segment explants of clonal apple rootstocks MM06 and MM111. The results have suggested that this protocol may be made commercially viable. There are four main steps in protocol developed *viz.*, culture establishment, shoot proliferation, rooting and acclimatization. The clonal apple rootstocks explants should be sterilized before culturing with 0.1 per cent $HgCl_2$ and could be inoculated on MS medium supplemented with plant growth regulators (cytokinins and auxins) for establishment, proliferation and rooting. The survival of the tissue culture raised plantlets of clonal apple rootstocks MM106 and MM111 can be increased in a soil medium containing sand: soil: vermiculite: FYM :: 1: 1: 1: 1 under controlled conditions. However, some works needs to be intensified to make the developed protocol commercially feasible by working out the nutritional and cultural requirement for the tissue culture plants in the field. It is estimated that using this protocol it would be possible to produce approximately plantlets from one initial explant of mature clonal apple rootstocks MM106 and MM111 at a cost of ₹ 44.56 and ₹ 36.95, respectively. The results obtained would be very useful for mass multiplication of clonal apple rootstocks under local condition and may provide guidelines for setting up commercial unit of propagation of these clonal apple rootstocks.

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CERTIFICATE- IV

Certified that all necessary corrections as suggested by the external examiner and advisory committee have been duly incorporated in the thesis entitled **"Propagation Studies on Clonal Rootstocks of Apple (*Malus* × *domestica* Borkh.)"**, submitted by **Mr. Manmohan Lal,** Registration No. **J-16-D-285-A**.

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