Influence of Plant Growth Regulators, Genotypes and Explants on Micropropagation of Gwarpatha [*Aloe vera* (L.) Burm.]

ग्वार पाठा {*एलो वेरा* (एल.) बर्म} के सूक्ष्म प्रवर्धन पर पादप वृद्धि नियामकों, जीनप्रारूपों एवं कर्तोतकों का प्रभाव

Divya Dixit

Thesis

Master of Science in Agriculture

(Plant Breeding and Genetics)

2019

Department of Plant Breeding and Genetics
S. K. N. COLLEGE OF AGRICULTURE, JOBNER
S. K. N. AGRICULTURE UNIVERSITY, JOBNER – 303329

Influence of Plant Growth Regulators, Genotypes and Explants on Micropropagation of Gwarpatha [*Aloe vera* (L.) Burm.]

ग्वार पाठा {*एलो वेरा* (एल.) बर्म} के सूक्ष्म प्रवर्धन पर पादप वृद्धि नियामकों, जीनप्रारूपों एवं कर्तोतकों का प्रभाव

Thesis

Sri Karan Narendra Agriculture University, Jobner

in partial fulfillment of the requirement for

the degree of

Master of Science in Agriculture

(Plant Breeding and Genetics)

By

Divya Dixit

2019

Sri Karan Narendra Agriculture University, Jobner

S.K.N. College of Agriculture, Jobner

CERTIFICATE - I

Dated: _____2019

This is to certify that **Ms. Divya Dixit** has successfully completed the comprehensive examination held on 20/05/2019 as required under the regulation for **Master's degree**.

(DHIRENDRA SINGH)
Professor & Head
Department of Plant Breeding and Genetics
S.K.N. College of Agriculture,
Jobner

Sri Karan Narendra Agriculture University, Jobner
S.K.N. College of Agriculture, Jobner

CERTIFICATE - II

Dated: _____2019

This is to certify that the thesis entitled **"Influence of Plant Growth Regulators, Genotypes and Explants on Micropropagation of Gwarpatha [*Aloe vera* (L.) Burm.]"** submitted for the degree of **Master of Science** in Agriculture in the subject of **Plant Breeding and Genetics** embodies bonafide research work carried out by **Ms. Divya Dixit** under my guidance and supervision and that no part of this thesis has been submitted for any other degree. The assistance and help received during the course of investigation have been fully acknowledged. The draft of the thesis was also approved by advisory committee on 04/06/2019.

(DHIRENDRA SINGH)
HEAD
Department of Plant Breeding and Genetics
S.K.N. College of Agriculture, Jobner

(M. L. JAKHAR)
Major Advisor

(G. S. BANGARWA)
DEAN
S.K.N. College of Agriculture, Jobner

Sri Karan Narendra Agriculture University, Jobner
S.K.N. College of Agriculture, Jobner

CERTIFICATE - III

Dated: _____2019

This is to certify that the thesis entitled **"Influence of Plant Growth Regulators, Genotypes and Explants on Micropropagation of Gwarpatha [*Aloe vera* (L.) Burm.]"** submitted by **Ms. Divya Dixit** to the Sri Karan Narendra Agriculture University, Jobner in partial fulfillment of the requirements for the degree of **Master of Science** in the subject of **Plant Breeding and Genetics** after recommendation by the external examiner, was defended by the candidate before the following members of the examination committee. The performance of the candidate in the oral examination on her thesis has been found satisfactory. We therefore, recommend that the thesis be approved.

(M. L. JAKHAR)
Major Advisor

(D. K. GOTHWAL)
Advisor

(SHAILESH GODIKA)
Advisor

(MAHESH DATT)
Director Education Nominee

(DHIRENDRA SINGH)
HEAD
Department of Plant Breeding and Genetics
S.K.N. College of Agriculture,
Jobner

(G. S. BANGARWA)
DEAN
S.K.N. College of Agriculture,
Jobner

APPROVED

DIRECTOR EDUCATION
Sri Karan Narendra Agriculture University, Jobner

Sri Karan Narendra Agriculture University, Jobner
S.K.N. College of Agriculture, Jobner

CERTIFICATE - IV

Dated:…../…../2019

This is to certify that **Ms. Divya Dixit** of the Department of **Plant Breeding and Genetics**, S.K.N. College of Agriculture, Jobner has made all corrections /modifications in the thesis entitled **"Influence of Plant Growth Regulators, Genotypes and Explants on Micropropagation of Gwarpatha [*Aloe vera* (L.) Burm.]"** which were suggested by the external examiner and the advisory committee in the oral examination held on …../…../2019. The final copies of the thesis duly bound and corrected were submitted on …../…../2019 and forwarded herewith for approval.

(M. L. JAKHAR)
Major Advisor

(DHIRENDRA SINGH)
HEAD
Department of Plant Breeding and Genetics
S.K.N. College of Agriculture, Jobner

(G. S. BANGARWA)
DEAN
S.K.N. College of Agriculture, Jobner

APPROVED

DIRECTOR EDUCATION
Sri Karan Narendra Agriculture University, Jobner

ACKNOWLEDGEMENT

*I wish to convey my benevolence to my major advisor **Dr. M. L. Jakhar**, Professor, Plant Breeding and Genetics, S.K.N. College of Agriculture, Jobner. I whole hearty extent my gratitude to him for giving me full freedom to do my research work. He has always showed optimism on my research with him novel ideas. He did not only guide me in my research endeavour but moulded me in always of my life.*

*I am grateful to my advisory committee members **Dr. D. K. Gothwal**, Professor, Department of Plant Breeding and Genetics, **Dr. Shailesh Godika**, Professor and Head, Department of Plant Pathology and **Dr. Mahesh Datt**, Professor, Department of LPM (Director Education Nominee), for their help and suggestions during entire period of investigation.*

*I take it to be my proud privilege to express my deep sense of gratitude, respect and esteemed reverence to **Dr. Dhirendra Singh**, Professor and Head, Department of Plant Breeding and Genetics*

*I express my sincere thanks to **Dr. B. K. Sharma**, Director Education, S.K.N. Agriculture University, Jobner for providing necessary guidance in the venture. I am highly grateful to **Dr. G.S. Bangarwa**, Dean, S.K.N. College of Agriculture, Jobner for his support to academic pursuits and the facilities provided to complete the present research work.*

*I feel great pleasure to express my deepest feeling and I offer my special thanks to **Dr. Kiran Gaur**, Assoc. Professor, Department of Statistics, **Dr. Varsha Kumari, Mr. Ram Kunwar** and **Dr. Manohar Choudhary** Asstt. Professors, Department of Plant Breeding and Genetics and for their valuable guidance, care, kind support and moral boost up during my research period.*

I am grateful to non-teaching staff members and laboratory staff members, Hari Ram, Jagdish Jakhar and Jiva Ji, Department of Plant Breeding and Genetics, SKNAU, Jobner for their help during my research period.

I wish to express my special thanks to my senior Ravi Kumar,Rajani Verma and Komal Shekhawat. I am especially thankful to my friends Aditya, Versha, Bharti, Shivani, Bhumika, Chanderkanta, Pukh Raj and Manoj.

*Words are inadequate to express illimitable veneration, adoration, indebtness and gratitude towards my parents whose inspiration, blessings, everlasting love and innumerable sacrifice have encouraged me in every step of my life, my beloved parents, father **Mr. Pramod Sharma**, mother **Mrs. Ranjana Sharma,** grandfather **(late) Sh. G.L. Sharma**, No words can express my love and affection to my elder sister **Dr. Khushboo Dixit** and my loving younger brother **Ishan Dixit**. They are the most important people in my world and I dedicate this thesis to them.*

Place: Jobner
Date: ____/____/2019 ***(Divya Dixit)***

LIST OF CONTENTS

LIST OF TABLES

Cont….

LIST OF FIGURES

Cont…..

ABBREVIATIONS/SYMBOLS

S. No.	Name	Symbols
1	6 - Benzyl- aminopurine	BAP
2	Indole-3- acetic acid	IAA
3	Kinetin	Kn
4	Napthalene acetic acid	NAA
5	Polyvinylpyrrolidone	PVP
6	2, 4-Dichlorophenoxy acetic acid	2, 4-D
7	Murashige and Skoog	MS
8	Thidiazuran	TDZ
9	Adenine sulfate	Ads
10	Hydrochloric acid	HCl
11	Mercuric chloride	$HgCl_2$
12	Plant growth regulator	PGR
13	Ultravoilet	UV
14	High efficiency particulate filter	HEPA
15	Sodium hydroxide	NaOH
16	Degree centigrade	^0C
17	Litre	l
18	Weight/Volume	w/v
19	Mili gram/litre	mg/l
20	Microgram	µg
21	Gibberelic acid	GA_3
22	Indole 3-butyric acid	IBA
23	Woody Plant medium	WPM
24	Centi meter	cm
25	Variety	Var.
26	Silver nitrate	$AgNO_3$
27	Cultivar	cv.
28	Figure	Fig.
29	Micro molar	µM
30	Isopentenyl adenine	2iP
31	Zeatine riboside	ZR
32	N-(2-Chloro-4-pyridyl)-N-phenyl urea	CPPU

Chapter- 1

INTRODUCTION

In India, tissue culture research began nearly six decades ago with thefirst report on production of test tube fertilization. Plant cell and tissue culture is defined asthe capability to regenerate and propagate plants from single cells, tissues and organs understerile and controlled environmental conditions (Murashige and Skoog, 1962).Tissue culture methods have also beenemployed to study the basic aspects of plant growth, metabolism, differentiation andmorphogenesis and provide ideal opportunity to manipulate these processes. Advancementin tissue cultural methodology led many recalcitrant plants amenable to *in vitro* regenerationand to the development of haploids, somatic hybrids and pathogen free plants (Gupta *et al.,* 2014).

Gwarpatha (*Aloe vera*L. syn. *Aloe barbadensis*Miller) belongs to family *Asphadelaceae*(Souza and Lorenzi 2005). It is an ancient, semi tropical medicinal plant indigenous to Africa, Madagascar and Arabia and introduced plant in India (Natali *et al*., 1990*,* Adams *et al.,* 2000, Campestrini*et al.,* 2006 and Nejatzadeh*et al*., 2012).Thisgenus has more than four hundred species of flowering succulent plants. *Aloe vera*L. is coarse looking evergreen perennial plant, grows up to 80-100 cm in height, with a strong fibrous root and atlarge stem supporting a rosette of narrow lanceolate leaves. The leaves are whitish green on both sides and bear spiny teeth on the margins. The yellow to orange drooping flowers produced in an inflorescence of 90-110 cm tall, each flower is pendulous, with yellow tubular corolla 2-3 cm and hermaphrodite.

Common species of *Aloe vera*around the world are *Aloe barbadensis*, *Aloesaponaria*, *Aloechinensis*, *Aloearborescens,Aloe variegata,Aloeferox*, *Aloelatifolia,* of these most popular is *Aloe barbadensis* and *Aloearborescens*. However, only*Aloe barbadensis* Mill.is grown today commercially, which has most therapeutic value and referred to as “True *Aloe*”. It is commonly known as “Medicine Plant”, “Burn Plant” , “First Aid Plant” , “Miracle Plant”, “Lily of Desert”, and “Ghritkumari” in Ayurveda (Tanabe and Horiuchi, 2006).*Aloe vera*L. has valuable medicinal properties and is commercially used in pharmaceuticals, cosmetics, food industries as nutraceuticals and for many herbal preparations. There are about more than 40 *Aloe*based formulations being marketed in the global market. The *Aloe vera*plant contains the important antioxidant vitamins (A, C and E), B (thiamine), niacin, B_2 (riboflavin), B_{12} (cobalamin), choline and B_9(folic acid) (Jayakrishna*et al.,* 2011), which prevent damages caused by free radical and reduces risk of chronic diseases. Natural antioxidants of *Aloe vera*increase the antioxidant capacity of the plasma and reduce the risk of certain diseases such as cancer, heart diseases and stroke (Prior *et al.,* 2000). The secondary metabolites like phenolics and flavonoids from plants have been reported to be potent free radical scavengers (Mathew and Abraham, 2006).

Aloe is fond of warmth and light, even being exposed in sunshine about two months, it will not die and onlypresents no continuous growth. On the contrary, it is afraid of cold, shady and humidity. It will be frozen and cannot grow when environmental temperature is below -10ºC, if it grows in pond soil for long time, its roots and leaves will be harmed seriously and become necrotic gradually. More interestingly,*Aloe* shows strong resistance to drought, salt and poor soil, certainly, it can grow better in loose and fertile soil

than in consolidated and nutrient deficient soil. Generally,*Aloe*can blossom after growing about 2 to 3 years nevertheless, its proliferation is mainly accomplished by tillering in root due to pollen abortion (Xiaohongand Jun, 2010).

Today also *Aloe vera* is one of the most studied plants worldwide. Actually, there is hardly any plant that can display such a great variety of scientifically proven active pharmaceutical ingredients (about 200) whose unique combinations properties generate a broad spectrum of activity (Davis *et.al*., 1988).

Internationally, *Aloe vera*is grown largely in South Texas of United States of America, India, Mexico, Central America, Australia and Africa. In India it is cultivated in Rajasthan, Gujarat, Haryana, Andhra Pradesh, Tamil Nadu and Maharashtra. In Rajasthan *Aloe vera* is cultivated in Churu, Sikar, Jaipur, Ajmer, Bikaner, Barmer, Jaisalmer, Jodhpur, Kota, Hanumangarh and Jhunjhunu districts.

In nature (*in vivo)*, *Aloe vera*is propagated through lateral buds which are slow, very expensive and low income practice (Meyer and Staden, 1991). Sexual reproduction by seeds due to male sterility in *Aloe vera* is almost ineffective and vegetative propagation through lateral shoots only possible during growing seasons (Keijzer and Cresti, 1987). However, the number of lateral shoots/donor plant is low and so it is difficult to plan in a rational basis a production system in commercial scale for obtaining plant propagation materials. Generally it is known that 3 to 4 lateral shoot/donor plant/year are produced in conventional system, which are not sufficient to meet the demand of pharmaceutical industries because for normal cropping it requires the density range of 18000-20000 plants /hectare.

In addition to this uncontrolled collection and sale of large quantities of *Aloe barbadensis* plant material lead to destruction of this species in nature. Local communities, traditional medicinal herbalists and herbal medicine vendors generally collect *Aloe* leaves from natural plantations leaving their propagation unattended, which is a serious problem.The technique of tissue and organ culture is used for rapid multiplication of plants, for genetic improvement of crops, for obtaining disease free clones and for preserving valuable germplasm (Bhojwani and Razdan,1992). One of the major applications of plant tissue culture is micropropagation or rapid multiplication. Compared to conventional propagation, micropropagation has the advantage of allowing rapid propagation in limited time and space.To overcome slow propagation rate, micropropagation will be a very useful technique for mass multiplication of *Aloe*.

Thus large scale plantation of *Aloe vera* through *in vivo* system of plantlet multiplication is insufficient to meet the requirements of farmers and pharmaceutical industries demand (Aggarwal and Barna, 2004 and Bhandari *et al*., 2010). Therefore, there is a need to develop suitable and alternative method for large scale propagation like *in vitro* propagation for rapid plant multiplication to meet the demand of farmers and pharmaceutical industries (Natali *et al*., 1990, Roy and Sarkar, 1991 and Abrie and Staden, 2001).

Micropropagation using stem and lateral shoot pieces of *Aloe vera*L. had already been proved successful (Natali *et al*., 1990, Meyer and Staden, 1991, Roy and Sarkar, 1991 and Aggarwal and Barna, 2004). However, source of explants, size, age, genotype, media composition, culture conditions, phenolic content of explants, exogenous supply of the plant growth regulators and media

discoloration greatly affect shoot regeneration from different genotypes of the same species. Therefore, the present study aimed to develop an alternative protocol for rapid and high frequency *in vitro* propagation of *Aloe vera*L., looking through its increasing demand for pharmaceutical industry at global level.

Considering the promises and limitations of conventional propagation approach, the present study has been undertaken toexplore the potential of shoot bud proliferation under influence of different plant growth regulators, genotypes and explants with following objectives:

(i) To determine the optimum level of different plant growth regulators for rapid shoot multiplication of *Aloe vera.*

(ii) To study the effects of genotypes and explants on micropropagation of *Aloe vera.*

(iii) To standardize protocol for rooting and hardening of micropropagated plantlets.

Chapter 2

REVIEW OF LITERATURE

Micropropagation is one of the innovative methods of asexual propagation, which proved to be effective for *in vitro* propagation of medicinal, endangered and horticultural plants (Debergh and Read, 1991). It is the practice of rapid multiplication of stock plant material to produce a large number of progeny plants, using modern plant tissue culture methods. It is used to multiply novel plants, such as those that have been genetically modified or bred through conventional plant breeding methods and to provide a sufficient number of plantlets for planting from a stock plant which does not produce seeds or does not respond well to vegetative reproduction.

In micropropagation cultures are started with very small pieces of plants (explants) and thereafter small shoots orembryos are propagated (hence the term‘micropropagation’ to describe the *in vitro* methods).Only a small amount of space is required to maintainplants or to greatly increase their number. Propagation is ideallycarried out in aseptic conditions. Through micropropagtion we may produce plants free from specificviruses and diseases in large numbers. Micropropagation may facilitate the flexible adjustment of factors influencingvegetative regeneration such as nutrient, growth regulator levels, photoperiod and temperature. This may enable newlyselected varieties to be made available quickly and widely and numerous plants to be produced in ashortwhile. The technique is very suitable when highvolume production is essential. Slow growing plants are possible to be cloned rapidly with less energy and space requirement for watering weeding and spraying etc. (George *et al.,* 2008).

Plant cell and tissue culture is defined as the capability to regenerate and propagate plants from single cell, tissue and organ

under sterile and controlled environmental conditions (Murashige and Skoog, 1974). In India, tissue culture research began nearly five decades ago with the first report on production of test tube fertilization (Kanta and Maheshwari, 1963).Plant tissue culture comprises a range of technologies integrated to commercial plant production, industries and research investigations (Smith, 1994). History of plant tissue culture techniques and their development are useful and integrated tools of modern plant biotechnology has been reviewed and discussed by Gautheret (1985), Thorpe (1990), Bhojwani and Razdan (1996), Thorpe (2000) and Vasil (2002). Tissue culture techniques are now being widely applied for improvement of field crops, forest, horticultural and plantation crops for increased agricultural and forestry production. Today, tissue culture technology is being exploited mainly for large scale production or micropropagation of elite planting material with desirable characteristics. This technology has now been commercialized globally and has contributed significantly towards the enhanced production of high quality planting material.

Inmicropropagation major role played by plant growth regulators, which includes naturally occurring plant hormones such as IAA, gibberellins, zeatin, ABA and ethylene etc. and also a number of synthetic chemicals that affect or control growth and development in plants. Each type of plant growth regulator has a wide range of physiological effects in different plants. These effects are determined by the kind of the growth regulator, its concentration, the presence or absence of other growth regulators, genetic makeup and the physiological status of the donor plant and kind of explants. The same physiological response in different tissues even of the same plant may require different growth regulator(s) or different combinations of growth regulators. Synergism and quantitative interaction of two or more growth regulators are of common occurrence. Finally, a growth regulator that elicits a positive response in a given tissue at a given

concentration may inhibit the same physiological response when used at higher concentrations.

Auxins, ABA, cytokinins, ethylene, and gibberellins are commonly recognized as the five main classes of plant hormones. Auxins, cytokinins, and auxin-cytokinin interactions are usually considered to be the most important for regulating growth and organized development in plant tissue and organ cultures, as these two classes of hormones are generally required. However, ABA, ethylene, gibberellins and other hormone like compounds have regulatory roles which must not be ignored in culture systems.Synthetic compounds that act like natural plant hormones are called "plant growth regulators" (Davies, 1995). Many such plant growth regulators have been discovered with a biological activity which equals or exceeds that of the equivalent endogenous hormones. In addition to these useful compounds, there are now quite a number of chemicals which interfere (generally inhibit) with the synthesis, transport or action of endogenous hormones. These inhibitors are extremely helpful in the study of the role of plant hormones in *in vitro* cultures.

Auxins exert a strong influence over processes such as cell growth expansion, cell wall acidification, initiation of cell division, organization of meristems giving rise to either unorganized tissue (callus) or defined organs (generally roots) and promote vascular differentiation. In organized tissue, auxins appear to be key players in maintaining apical dominance, affecting abscission, promoting root formation, tropistic curvatures, delaying leaf senescence, and fruit ripening (Addicott, 1982, Sabater, 1985, Chandler and Thorpe, 1986, Liu and Reid, 1992, Aloni, 1995 and Tamas, 1995).Two major properties of cytokinins that are useful in culture arestimulationof cell division (often together with auxins) and release of lateral bud dormancy. They can also induce adventitious bud formation (Fabijan *et al*., 1981 and Krikorian, 1995).Cell division is regulated by the joint

action of auxins and cytokinins, each of which influences different phases of the cell cycle. Auxins affect DNA replication, whereas cytokinins seem to exertsome control over the events leading to mitosis (Vesely*et al*., 1994) and cytokinesis. Thus auxin and cytokinin levels in cultures need to be carefully balanced and controlled. In intact plants, cytokinins promote lateral bud growth, leaf expansion, retard leaf senescence, promote chlorophyll synthesis and enhance chloroplast development (Kuhnle*et al*., 1977 and Nooden and Leopold, 1988). Gibberellins can promote flowering (particularly in species that require long days and/or cold), cone initiation in some conifers, seed germination and stem elongation (by increasing cell division and elongation). The ability to promote bolting and stem elongation can be exploited *in vitro* for the elongation of the shoots of woodyspecies before rooting. Some GA effects are caused by increases or decreases in the biosynthesis and activity of specific enzymes (e.g., increases in levels of aleurone hydrolytic enzymes).ABA is important in seed maturation, as it induces the synthesis of storage proteins in developing seeds. It acts antagonistically to gibberellins in many systems. ABA, along with ethylene and jasmonic acid, aids in defense against insect wounding. With ethylene, ABA is intimately involved with plant responses to a wide range of environmental stresses. Depending upon the time after subculture, ethylene can stimulate or inhibit growth and organogenesis in *in vitro* cultures (Huxter*et al*., 1981). Ethylene can specifically affect growth of callus and suspension cultures, stem and root elongation, axillary and adventitious bud formation, rooting and embryogenesis.

In 1957 Skoog and Miller put forth the concept of hormonal control of organ formation. They showed that the differentiation of roots and shoots in tobacco pith tissue cultures was a function of the auxin cytokinin ratio and that organ differentiation could be regulated by changing the relative concentrations of the two substances in the

medium, high concentrations of auxin promoted rooting, whereas high levels of cytokinin supported shoot formation. At equal concentrations of auxin and cytokinin the tissue tended to grow in an unorganized fashion. This concept of hormonal regulation of organogenesis is now applicable to most plant species.

The most common plant growth regulators used in the tissue culture and micropropagation includes auxin, NAA and cytokinin, BAP (Choudhary*et al*., 2011). The type andconcentration of plant growth regulators added to basal or half strength MS and/or other growth media affect regeneration of plants. The commonest plant growth regulators used in plant tissue culture and micropropagation are 2,4-D, BAP, IAA, IBA, and NAA applied alone or in various combinations. Other inputs may include sucrose (as carbon source), agar (as solidifying agent), antioxidants such as citric acid, ascorbic acid, PVP and activated charcoal (to reduce excessive browning of explants and medium). Micropropagation responses of explants of various plants are also affected by pH, photoperiod, light intensity, RH, and temperature (Hosseini and Parsa, 2007, Hashemabadi and Kaviani, 2008, Narayana, 2008 and Choudhary*et al*., 2011).

2.1 ROLE OF PLANT GROWTH REGULATORS IN MICROPROPAGATION OF *ALOE VERA*

Due to slow multipilication rate of plantlets under natural conditions and increasing demand of *Aloe vera* for its pharmaceutical uses its required demand of plantlets for large scale plantation cannot be fulfilled, hence there is a need for mass propagation of this plant through *in vitro* methods (Kumari and Naseem, 2015).

Micropropagation using stem and lateral shoot pieces of *Aloe vera*L. had already beenproved successful. However, source of explants, size, age, genotype, mediacomposition, culture conditions and phenolic content of explants and media discolorationgreatly affect

shoot regeneration from different genotypes of the same species.Also, the presence of the plant growth regulators is necessary for this purpose.

Corneanu*et al.* (1994) carried out micropropagation of *Aloe*by culturing fragments from auxiliary shoots on MS medium without growth regulators (the presence of which was found to inhibit the first stage of development). For the second stage of development involving the newly formed plantlets, the presence of a magnetic fluid in the culture medium stimulated secondary shoot production, general plant development and rhizogenesis.

Richwine*et. al*. (1995) reported the induction of shoot cultures of*Aloe, Gasteria*and*Haworthia*species from immature inflorescence. Shoots were initiated on amodified MS medium containing zeatin and later maintained on medium containing zeatin and BAP.

Abrie and Staden (2001) reported a rapid propagation protocol for the highly endangered *Aloe polyphylla*(Schonland ex Pillans). Plantlets were cultured on MS medium containing BAP only, or a combination of BAP and NAA. After initial problems of browning, the explants rapidly formed auxiliary and adventitious buds. Maximal shoot formation was obtained on MS medium containing 1.0 mg/l BAP. Some shoots rooted spontaneously on MS medium, but the rooting percentage was improved with a 0.5 mg/l IBA supplement. Rooted plantlets were acclimatized to greenhouse conditions. The success of this project indicates that micropropagation can be a useful tool in the conservation of this endangered species of the genus *Aloe.*

Chaudhuri and Mukundan (2001) cultured shoot tips of *A. vera*on MS medium containing 3 per cent sucrose and supplemented with AdS,BAP, IAA and IBA alone or in combinations. Leafy shoots differentiated in almost all the treatments. Sixty days after culture, 2-3 cm shoots were subcultured for 4 weeks on half strength MS medium supplemented with 1.0 mg/l IAA. The formation of multiple shoots *in*

vitro was due to the presence of cytokinin and auxin. However, the presence of only auxin or only cytokinin in the culture medium resulted in root or callus formation. The optimal medium for maximum shoot formation was MS medium + 10.0 mg/l BAP + 160.0 mg/l AdS + 0.1mg/l BAP. Leafy shoots cultured on half strength MS medium + 1.0 mg/l IAA produced as many as 20 shoots/explant. The fresh gel exudate from both the control plants and tissue culture derived acclimatized plants contained about 1.0 per cent dry matter. The average level of soluble solids was 0.6-0.7 per cent and fresh weight and fiber content was 0.075 per cent. The gels from both types of plants were similar and mainly comprised of reducing sugars (mannose and glucose in 3:1 ratio).

Chukwujekwu*et al*. (2002) establishedrapid tissue culture protocol for the highly endangered *Aloe polyphylla*(Schonland ex Pillans). Shoot cultures of *Aloe polyphylla*were initiated from young shoot explants of *in vitro*grown plants. The basal medium was MS medium supplemented with100mg/l myo inositol, and 30 g/l sucrose. Differentcytokinins (Kn, zeatin and BAP) singly or in combinationwith auxins (IBA and NAA) were tested for shootproliferation activity. All the cytokinins gave good shootproliferation. The optimal concentrations for shoot proliferationof each of the cytokinins tested were: zeatin(0.5mg/l), Kn(1.5 mg/l) and BAP (1.5mg/l). In combinationwith auxins, the optimal combinations wereKn/NAA (2.0/0.1mg/l), Kn/IBA (1.5/1.0mg/l),zeatin/IBA (1.0/0.5 mg/l), zeatin/NAA (1.0/1.0 mg/l),BAP/IBA (1.0/1.0mg/l) and BAP/NAA (1.5/0.1mg/l).Although it gave the highest number of shoots perexplant, BAP (1.0–3.0mg/l) induced hyperhydricity. Temperature and sucrose also influenced shoot proliferation.The optimal temperature was 25°C, while 30 g/l was the optimal concentration of sucrose. Plants rooted well in plant growth regulator free MS medium. Amongst the potting mixtures tested,

soil:sand:vermiculite(1:1:1 v/v/v) was the best, with a 98 per cent plantlet survival.

Wenping*et al*. (2004) and Liao *et al.* (2004) reported good combinations of growth regulators in proliferating shoot were BAP 2.0 mg/l and Kn 0.2 mg/l (95.30 per cent) followed by BAP 2.0 mg/l (90.91 per cent). MS medium containing BAP and NAA was found to be the best medium in *aloe*micropropagation.

Eufrocinio and Malasa (2005) placed nodal explants of *A. barbadensis* in MS medium containing different levels of Kn or BAP to induce multiple shoot formation. The best treatment for multiple shoot induction was 1.0 mg/lBAP, which produced an average of 11 shoots per explant. Individual shoots from the multiple shoot clumps were taken and transferred in MS medium containing different levels of IAA, NAA and IBA for rooting. The best auxin for root formation was NAA, with an optimum concentration of 0.10 mg/l. Rootedplantlets were successfully transferred in the field after hardening.

Velcheva*et al.* (2005) reported *invitro* regeneration in *Aloe arborescens* using young inflorescences as explants. Different phytohormone combinations of TDZ, BAP, 2iP, ZR, CPPU and Kn with or without ancymidol were examined in order to induce plant regeneration. Efficient shoot regeneration was initiated on MS medium supplemented with BAP or TDZ. MS medium enriched with 19.6, 22.2 µM BAP and 3.92 µM ancymidol promoted organogenesis enabling 87.3 per cent of the explants to regenerate 6.04 ± 1.79 shoots/explant. Subsequent shoot elongation and plant regeneration were strongly affected by the medium composition used for shoot induction. Optimal elongation (three to four shoots per explant) was obtained on MS containing only 4.4 µM BAP. Rooting was induced on MS media lacking growth regulators. Histological analysis revealed that the

initiated shoot originated from the receptacle tissue surrounding the residual vascular tissue of the flower buds.

Tanabe and Horiuchi (2006)reported that media containing BAP and sucrose promoted shoot growth. A medium combination with 2.0 g/l sucrose + 2.2 µMBAP showed regeneration of a new shoot in 80 per cent of its cultures in 6 weeks. Exclusion of BAP or sucrose resulted in regeneration in 40 per cent or less of the cultures. Although BAP plays an important role in shoot initiation.

Ahmed *et al.* (2007) standardized an efficient micropropagation method using shoot tip explants in *Aloe vera*(L.). The process involves subsequent *in vitro* morphogenesis and rooting of the *in vitro* proliferated shoots and transplantation of regenerated plants under *exvitro*condition. Shoot proliferation was found best in MS medium containing BAP 2.0 mg/l, Kn 0.5 mg/l and NAA 0.2 mg/l. Maximum shoots (98.96 per cent) were proliferated in this media composition. This media composition is best comparing to other treatment used in this study. Highest shoot number per explant was also achieved in the same medium within 5 weeks. In case of adventitious rooting, MS medium containing NAA 0.2 mg/l and NAA 0.5 mg/l was found to be the best. Maximum rooting (80.25 per cent) and highest number of roots per culture (6.71) were obtained in this media composition. After transplanting, the 20 days old rooted shoots into mixture of garden soil, compost and sand (2:1:1), 80 per cent of survivability after 5 weeks was achieved. Regenerated plants after acclimatization were transferred to field and they showed 82 per cent survival. The regenerated plants were morphologically similar to the mother plants.

Hosseini and Parsa (2007) reported maximum shoot induction in *Aloe vera* on the MS medium supplemented with 1.0 mg/l BAP+1.0 mg/l Kn. Root induction was achieved on hormone free MS medium

and upon transferring the plants into soil and the survival rate was 83 per cent.

Hashemabadi and Kaviani (2008) observed that regeneration of *Aloe vera* in nature (*in vivo*) is too slow and insufficient to meet the industry demand. Therefore, it is necessary to use *in vitro* propagation for rapid plant production. Explant used for the *in vitro* culture was shoot tip. The shoot tip explants were disinfected with 2 per cent NaOCl and washed thoroughly with sterile water. Then, explants were placed on solid MS medium with the addition of various concentrations of BAP and NAA. After 8 weeks, the best proliferation of shoot per explant and rooting on the medium supplemented with 0.5 mg/l BAP + 0.5 mg/l NAA. The rooted plantlets were gradually acclimatized in plastic pots containing garden soil and finally transferred to the field.

Singh *et al.* (2009)reported the development of an efficient method for rapid clonal propagation by shoot proliferation from auxiliary meristem(s) of selected germplasm of *Aloe vera*. Explants were pretreated with 0.1 per cent aqueous solution of both streptomycin and bavistin separately, each for 15 min. These were surface sterilized with 0.1 per cent aqueous solution of mercuric chloride ($HgCl_2$) for 4–5 minutes and washed several times with autoclaved double distilled water. These were kept in a chilled, sterile antioxidant (200.0 mg/l of ascorbic acid, 50.0 mg/l of citric acid, and 25.0 mg/l of PVP) solution and cultured on semi solid MS medium. The bud explants produced multiple (10.3 ± 0.675/explant) shoots on MS medium containing 13.32 µM of BAP and 100.0 mg/l of ascorbic acid, 50.0 mg/l each of citric acid and PVP, with 25.0 mg/l each of arginine and adenine sulphate as additives. The shoots were further multiplied by (a) repeated transfer to fresh MS medium with additives + 13.32 µMBAP and (b) subculturing on MS medium with a lower (4.44 µM) concentration of BAP. On MS medium containing 4.44 µM of BAP and additives, a maximum number (27.8 ± 0.63) of shoots were produced. In liquid MS medium with 4.44

µM of BAP, the rate of shoot multiplication increased and the vigour of the shoots improved. Hundred percent of the cloned shoots rooted under*in vitro* conditions on hormone free half strength MS salts containing 200.0 mg/l of activated charcoal at 32 ± 2°C. The cloned shoots treated with 2.46 mM of IBA or 2.473 mM of NAA for 5 minutes rooted under ex vitro conditions in the greenhouse. The rooted plants were hardened in the greenhouse and stored under a net house. The cloned plants were transferred under different field conditions at various sites in Western Rajasthan. These plants grew normally.

Hashem and Kaviani (2010) obtained maximum proliferation of shoot buds in shoot tip explants of *Aloe vera* on MS medium supplemented with 0.5 mg/l BAP + 0.5 mg/l NAA. The highest number of roots was obtained on medium containing 1.0 mg/l BAP + 1.0 mg/l NAA. The longest and thickest roots were achieved on medium supplemented with 1.0 mg/l IBA + 1.0 mg/l NAA. Minimum microshoots were obtained in control.

Kalimuthu*et al*. (2010) observed proliferation of shoot buds in shoot tip explant of *Aloe vera* on MS medium supplemented with 1.5 mg/l BAP and 50 mg/l Ads. Ninety per cent microshoots produced rooted plantlets within 12 days of culture on MS medium fortified with 1.0 mg/l of NAA. The plants were successfully acclimatized to shade house condition and transferred in the soil.

Jakhar*et al*. (2012) observed multiple shoot induction in MS medium supplemented with varying concentrations of BAP, Kn, NAA and 2,4-D either added singly or in combination. The cultures were incubated at 25 $\pm$ 2^0C under 14:10 photoperiod with light intensity of 3000 lux. Maximum shoot bud proliferation was achieved at 0.5 mg/l BAP.

Shekhawat *et al.* (2013) reported that auxiliary shoots (3.0–6.0 cm long) were appropriate explants for theestablishment of clonal

cultures of *Aloe vera*.Maximum response was observed on MSmedium with 3.0mg/lof BAP and additives.Repeated transfers of the mother explants were done on semisolid MS mediumwith 3.0mg/lBAP and additives. The *in vitro* produced shoots were multiplied on MS medium containing 1.0 mg/l BAP and 4 per cent sucrose. On MS + 1.0mg/lBAP,about 28 shoots differentiated per culture vessel. More than 30 shootsregenerated per culture vessel in liquid medium with 1.0mg/lBAP and additives. The growth of the shoots was rapid therefore, subculturingwasdone every fortnight to maintain optimum growth and rate of multiplication.

Gupta *et al.* (2014) *observed* the development of callus in the MS mediasupplemented with BAP for best regeneration in *Aloe vera*, IBA for root formation and NAA, was foundthe best media for root formation (0.5mg/l) were seen to grow onwards from the tenth day of culture and 90 per cent of root formation took place within a span of 3-4 weeks for maximumcallus induction. The maximum number of root and shoot produced was 4.8±0.53 with averagelength of 3.5±0.35cm.

Sahoo and Rout (2014) reported that BAP was more effective for shootbuddevelopmentthanKn and adenines in *Aloe barabadensis*. The shoot bud initiation was observed within 3–4 weeks of culture on MS mediumsupplemented with 2.0 mg/l BAP. Callusing was not observed in the basal end of the explants. Combination ofBAP + NAA favours more effective for shoot bud proliferationthan the Kn + NAA. Inclusion of 25 to 40 mg/l Adenines in culture medium enhances the rate of shoot bud proliferation. The inclusion of gibberelic acid (0.25–0.50 mg/l) inthe culture medium showed the maximum elongation ofshoots. The percentage of shoot bud regeneration was maximum(55.6 per cent) within 4–6 weeks of culture on MS mediumfortified with 2.0 mg/l BAP, 0.5 mg/l NAA, 40 mg/l adeninesulfate and 3 per cent sucrose. There was nosign of meristematic shoot bud proliferation when leaf explantswere cultured in

media without cytokinin. Further, increase in the concentration of either BAP or Kn alone or incombination had no effect on the rate of shoot bud development or multiplication.

Kumari*et al*. (2015) studied to assess the effect of different hormones like auxin and cytokinin on regeneration potential of rhizomatous stem and leaf segment used as explants of *Aloe vera*. Best shoot proliferation (6-7 shoots/ explants) was obtained on MS medium containing 2.5 mg/l BAP. Optimum growth of callus was achieved from rhizomatous stem and leaf segment explants on medium containing NAA+BAP + IBA (2.5+2.0 +0.5 mg/l). The best rooting of micro shoots were obtained on shoot regenerating medium containing both BAP and NAA, 2.5mg/l each.

Shukla *et al.* (2016) conducted micropropagation of *Aloe vera* using young shoot tip explants and observed best result of shoot proliferation when cultured on MS media with 0.2×10.0–3.0 mg/l IBA and 0.2×10.0–3.0 mg/l BAP. Addition of 0.2 mg/l of citric acid gave the best control of phenolic secretion during shoot proliferation. For shoot elongation and rooting, MS media with 2.0×10.0–3.0 mg/l BAP and 0.2×10.0–3.0 mg/l IBA gave best results.

Pugazhendhi and Sekar (2017) observed bud initiation in *Aloe vera.* MS medium was supplemented with BAP and NAA in combinations. The highest (84 per cent) bud initiation was observed when MS medium supplemented with 1.5 mg/l BAP + 0.1 mg/l NAA + 3 per cent sucrose in auxiliary shoots.

Surafel*et al.* (2018) reported MS media supplemented with 0.6 mg/l BAP and 0.2 mg/l of IBA, 1.0 mg/l BAP and devoid of hormone were optimum for *Aloe vera*establishment, multiplication and rooting respectively. The higher mean number of shoots (2.90) and multiplication factor (2.87) were observed on MS media with 0.2 mg/l IBA and 1.0 mg/l BAP respectively.

2.2 EFFECT OF EXPLANTS

Explants are small pieces of plant parts or tissues that are aseptically cut and used to initiate a culture in a nutrient medium.The type of explants, size, position, age, physiological state and the manner in which it is cultured can affect the initiation of the cultures and further morphogenetic response (Murashige and Skoog, 1974). Often there is an optimum size of explant suited to initiate cultures. Very small shoot tips or fragments do not survive well while it is difficult to decontaminate larger explants. Larger the sizes of the meristematic tissues are advantageous. Selecting juvenile parts of the plants yield better than older ones. The size of explant is also likely to influence the uptake of mineral salts irrespective of whether it is grown on liquid or solid medium (George and Sherrington, 1984).Types of explants are one of the important factors in optimizing the tissue culture protocol like leaf, petiole, cotyledonary leaf, hypocotyl, epicotyl, embryo, internode and root explant significantly effect on tissue culture process of plants (Khan *et al*., 1988, Sujatha and Mukta, 1996, Tyagi*et al*., 2001, Gubis*et al*., 2003, Alagumanian*et al*., 2004, Ali and Mirza, 2006 and Kumar *et al*., 2011). This may be due to the different level of endogenous plant hormones present in the plants parts.

Hirimburegama and Gamage (1995) obtained high rate of shoot proliferation from auxiliary and apical buds of *Aloe vera*by culturing on MS medium supplemented with 0.18 mg/l IAA and 2.25 mg/l BAP. Rooting was achieved on MS medium supplemented with 0.18 mg/l IAA and 0.22 mg/l BAP for 3 weeks. Plantlets were ready for transfer to soil within 8 weeks. Rooted plants were successfully acclimatized.

Fougat*et al*. (1997) cultured four types of explants (leaf, shoot tip, nodal segments from field grown trees and cotyledons from laboratory grown seedlings) of pomegranate cv. Ganesh on MS medium supplemented with various growths regulator combinations.

Exudation of phenolics was overcome by pretreating the explants for 2-3 days with varying concentrations of antioxidant compounds such as PVP and ascorbic acid. Callus growth and induction from cotyledon and leaf explants were best on MS medium supplemented with4.0 mg/l NAA, 2.0 mg/l Kn and 15 per cent coconut water.

Liao *et al.* (2004) achieved shoot multiplication from shoot tip explants by using MS medium supplemented with 2.0mg/l BAP + 0.3mg/l NAA while working on micropropagation of *Aloe vera.*

Hongxing Wang *et al.* (2004) reported the relationship between aloin accumulation of *Aloe vera* var. chinensis and the callus cultured by the roots, stems and leaves as explants. Results showed that on the MS medium with 1.0 mg/l NAA + 0.5 mg/l BAP, the differentiation degree of the callus induced from the leaves was in highest level, meanwhile the callus contained the most aloin. The aloin content was low in callus from stems. There was no aloin in callus from roots. It was found that on MS medium with 1.0 mg/l 2,4- D + 0.5 mg/l BAP, the callus differentiation was in low level and without aloin, no matter what organs were used.

Chaugule*et al.* (2005) observed callus induction in leaf segment and cotyledon of pomegranate in the MS media supplemented with BAP sand NAA. The minimum number of days for callus induction from the leaf segment was 8.8 days, whereas the cotyledon recorded was10 days. Callus initiation occurred at the cut ends and spreaded over the surface of the explants as a result of injury in all the treatments. Regarding callus weightandproliferation,theMSmediumcontainingNAA0.4mg/l+BAP 1.0 mg/l was found to be the best in both the explants. However, the callus derived from the leaf segment showed greater dry weight than that of derived from the cotyledon. The calluses were inoculated for shoot organogenesis and embryoid formation in different culture

media. However, the calluses derived from both the explants showed recalcitrant nature.

Benerjee *et al.* (2007) reported rapid clonal propagation through *in vitro* techniques using cotyledonary node as an explant in the high yielding, tikka susceptible (ICG 11337, AK 1224, ICGS 44, JL 24) and tikka immune (ICG 6284) varieties of *Arachis hypogaea* L. The cotyledonary nodes when cultured in the presence of various concentrations of BAP (1.0, 5.0, 10.0, 15.0, 25.0, 50.0 mg/l). BAP free control showed very little or no sign of multiplication in terms of multiple shoot, auxiliary branch and shoot bud formation. In the presence of higher concentrations of BAP, cultured explants showed the development of multiple shoots, auxiliaries branch and shoot buds. The regeneration of maximum number of shoots auxiliary branches and shoot buds from the excised cotyledonary nodes and their corresponding requirements for BAP differed across varieties, which might be due to variation at their genotypic levels.

Monge *et al.* (2008) reported leaf bases best explants for induction of callus in *Aloe barbadensis* Mill., cultured on MS medium supplemented with 2.5 mg/l 2,4-D + 2 mg/l BAP and 40.0 mg AdS. The highest number of shoots regenerated from callus cultures when, supplemented with 0.2 mg/l 2,4-D and 2 mg/l BAP.

Singh *et al*. (2010) cultured different explants *viz.,* shoot tips, nodal, intermodal and leaf segments of *Commiphoramukul*on MS medium supplemented with various concentration (0.5-2.0 mg/l) of auxins and cytokinins individually and in combinations. Result showed that nodal explant responded better in comparison to rest of the explants for bud break on the media supplemented with 2.0 mg/l BAP + 0.5 mg/l IAA.

Rathore *et al.* (2011) developed tissue culture method for high frequency plantlet regeneration from inflorescence axis derived callus

cultures of swe*et Al*oe genotype. Competent callus cultures were established on 0.8 per cent agar gelled MS basal medium supplemented with 6.0 mg/l of 2,4-D and 100 mg/l activated charcoal and additives. The callus cultures were cultured on MS medium containing 1.5 mg/l 2,4-D, 0.25 mg/l Kn and additives with 4 per cent carbohydrate source for multiplication and long term maintenance of regenerative callus cultures. The hundred per cent regenerated plantlets were hardened in the greenhouse and stored under an agro net house.

Abdi*et al*. (2013) studied on a method for mass propagation of *Aloe vera* by using different explants and different media with different plant growth regulators. Two type of explants (with and without sheath Type A and B respectively) Highest rate of shoot induction observed in MS medium supplemented with 0.2 mg/l NAA and 4.0 mg/l BAP in type A explants. Also, the highest shoot proliferation response obtained successfully by using MS medium containing 4 mg/l BAP.

Haque and Ghosh (2013) studied on nodal portion of rhizomatous stem of *Aloe vera*and cultured on MS medium supplemented with various cytokinin and *A. vera* leaf gel as organic supplement. Number of proliferated shoots per explant was increased along with the regeneration cycles and on MS medium supplemented with 2.5 mg/l BAP and 10.0 per cent (v/v) *Aloe vera*leaf gel, only 17.8 ± 0.35 shoots per explant were induced on 1stregeneration cycle whereas on 3rd regeneration cycle these number increase to 38.5 ± 0.44 shoots per explant on the same medium composition on the effects of different cytokinin types and concentrations on the explant for shoot induction up to three regeneration cycle.

Kalalbandi*et al.* (2014) studied micropropagation in Bhagawa variety of pomegranate undertaken using different explants from

fruiting mother plants. Shoot tips were found the best explants for culture establishment.

Bensaad and Milad (2015) reported that single node explants responded better than shoot tips based on number of growing shoots, number of leaf per explants and shoot length on WPM supplemented with4.5 µM BAP in pomegranate.

Suhasini*et al.* (2017) observed that second nodal segment recorded the highest aseptic culture establishment when explants were sterilized with mercuric chloride ($HgCl_2$) at 0.01 per cent for 3 minutes followed by incubation on full strength MS medium in pomegranate cv. Bhagawa.

Kiran *et al.* (2017) reported leaf tip and spine did not show any response whereas, apical shoot multiplied. The size of explants at time of culture was found critical for shoot initiation. Among the 3 explants, the apical bud explants gave the best results and were used for further experiments. MS medium with 2 mg/l BAP + 0.2mg/l NAA was found best for multiple shoot initiation from apical shoot. As they produced 5.0 mean number of shoots/explant with 92.85 per cent shoot initiation response. The similar results were obtained by Ahmad *et al*. (2007), Mehta (2013), Gupta*et al*. (2014) and Kumari*et al.* (2015).

2.3 EFFECT OF GENOTYPES

Physiological conditions of the mother plants, which are indirectly determined by the genotype of the plant exert considerable influence on the process of morphogenesis. The most actively growing plants are suitable for the selection of explants.

Genotype is one of the most important factors affecting regeneration (Kumar *et al*., 2011). Success of regeneration is predominantly dependent on the genotypes from which explants were obtained. Various species and cultivars exhibit different growth

responses in culture. It is now known that regeneration ability is genetically controlled. Thus a general survey for tissue culture response of various cultivars was undertaken with simple media, as complex media rich in growth regulators tend to favour of organogenesis of somatic tissue giving rise to callus of various ploidy levels.

Chandra *et al.* (1995) reported significant genotypic differences between hybrids variety and non hybrid variety of tomato in adventitious shoot bud formation from leaf explants culture. The frequency of adventitious shoot bud formation was higher in hybrid tomato than non hybrid tomato.

Richwine *et al.* (1995) reported the induction of shoot culture in *Aloe, Gasteria* and *Howarthia* species from immature inflorescence. Shoots were initiated on a modified MS medium containing zeatin and later maintained on medium containing Zeatin and BAP.

Schween and Schwenkel (2003) reported that genotypic differences between six genotypes of *Primula vulgaris* were achieved in callus induction rate, type of callus root formation during the callus phase, and shoot regeneration rate.

Tyagi and Prakash (2004) reported differences in rooting behaviour in male and female plants of jojoba. Female genotypes were more responsive as compared to male genotypes.

Sanatombi and Sharma (2008) reported influence of genotype on capsicum for direct organogenesis. The cultivar Umorok showed the best response while Meiteimorok, Haomorok, Mashingkha and Uchithi showed intermediate response and the cultivar Chiengpi was the least responsive.

Alezadeh *et al.* (2010) conductedthe studies on micropropagation of four genetically different grape rootstocks namely

Dogridge (*Vitischampini*), SO-4 (*V. riparia*× *V. berlandieri*), H-144 (*V. vinifera* × *V. labrusca*) and 3309 C (*V.riparia* × *V. rupestris*) to develop an optimized protocol and to compare *in vitro*behaviour of these genotypes.Least success (38.31 per cent) in culture establishment was observed for H-144 but it exhibitedbetter vegetative growth and rooting among genotypes i.e. higher shoot multiplication rate (12 microcuttingsper culture), highest rooting (87.7 per cent) and early root initiation (11.52 days). Addition ofactivated charcoal to the rooting medium was found beneficial with respect to enhancement of rootingand minimizing time to root initiation in different genotypes. Among the rootstock genotypes, 3309 Cwas found most responsive in terms of higher *ex vitro* plantlet survival (84.95 per cent) during hardeningand shorter duration required for *ex vitro* transfer. These results indicate the significant influence of different factors like culture medium and genotypeon the overall micropropagation of grape rootstocks.

Payghamzadeh and Kazemitadar (2011) reported optimization of culture medium for embryo culture of eight cultivars of walnuts. Immature embryos were cultured on DKW basal medium. Many cultivars were capable of supporting the conversion of plantlets from young embryos. It was observed that different cultivars had different requirement for growth regulators. Among different cultivars, the highest per cent of embryo germination were achieved in Chandler, Serr, Hartky, Rentegnomushak and local cultivars.

Mittal *et al*.(2015) repoted maximum percentage of shoot elongation in sugarcane genotype CoJ 83 (98.53 per cent) followed by CoJ 88 (92.57 per cent) and CoJ 64 (85.09 per cent) on the elongation medium supplemented with 2.0 g/l activated charcoal.

Gheisari and Miri (2017) observed that hormonal requirement for callus induction and direct bulblet regeneration of two lisianthus varieties was different.

Mangena (2018) reported that soybean cultivars TGX 1740-2F and TGX 1835-10E were highly recalcitrant. These cultivars showed consistent difficulties for callus and shoot proliferation under used culture conditions. Consecutively, the highest number of shoots was induced in soybean cv. Peking, LS 677 and LS 678. This was followed by cultivar Dundee and lastly, the two TGX varieties.

2.4 ROOT INDUCTION IN MULTIPLIED SHOOTS

Abrie and Staden (2001) observed that rooting response in *Aloe pollyphylla* was unpredictable and investigated the influence of the auxin IBA (0.5 mg/l) compared to the response on MS medium containing BAP (0.1 mg/l). After four weeks, 64.2 per cent of the plantlets had formed roots on the IBA containing medium, compared to only 21.4 per cent on the BAP containing medium. After eight weeks, this improved to 71.4 per cent and 64.4 per cent respectively. On both media, the roots appeared normal and turned yellow or brown at maturity.

Baksha*et al.* (2005) reported that root formation was induced in *in vitro* regenerated shoots of *Aloe barbadensis* by culturing them on half strength of MS supplemented with 0.5 to 1.5 mg/l of IBA or NAA or IAA. In the medium with 0.5 mg/l of NAA, roots began to emerge from the 10th day of culture and within a period of 23-28 days frequencies of root formation were 95 per cent. The highest number of roots per shoot was 4.8 ± 0.53 with an average length of 3.5 ± 0.35 cm. The roots that developed in the medium containing higher concentration (1.0-1.5 mg/l) of auxin were poor in quality.

Ahmed *et al.* (2007) reported that proliferating shoots of *Aloe vera* took maximum 7-8 weeks to attain the size suitable for rooting (>2 cm). The highest percentage of shoots that induced roots (80.25 per cent) was observed in MS medium supplemented with NAA 0.2 mg/l followed by IBA 0.2 mg/l. Effect of IAA in rooting was very poor. The highest number of root per culture (6.71) was found in MS medium containing NAA 0.2 mg/l.

Hashemabadi and Kaviani (2008) reported rooting in the presence of low concentrations of BAP and NAA in *Aloe vera*. They also revealed that there is a negative correlation between rooting and BAP concentration in the medium. The shoots showed good rooting on MS medium supplemented with 0.5 mg/l BAP + 0.5 mg/l NAA and 1 mg/l BAP + 0.5 mg/l NAA. The largest number of roots was obtained on medium supplemented with 0 mg/l IBA + 1 mg/l NAA (9.71) and the longest (8.75 cm) and thickest (4.3 cm) roots were achieved on medium supplemented with 1 mg/l IBA + 1 mg/l NAA.

Singh *et al.* (2009) reported rooting on hormone free half strength semi solid MS salts with 200.0 mg/l of activated charcoal. 100 per cent of the shoots rooted at 32 ± 2°C. Root induction in *Aloe vera* was observed after 10-12 days of inoculation. Cloned shoots also rooted under *ex vitro* conditions if treated with root inducing hormone (IBA/NAA) for 5 minutes. Treatment of shoots with 2.473 mM NAA for 5 minutes, more than 95 per cent of the shoots was rooted on soilrite. Root initiation was observed after 13-15 days of auxin treatment. Higher concentrations (more than 2.473 mM) of root inducing hormone (NAA) cause deterioration of shoot bases and no rooting was observed there. On lower concentrations of NAA (less than 2.473 mM), the percentage of rooting wasless and root induction was also delayed.

Nayanakantha*et al.* (2010) suggested that external application of auxin is not necessary for root induction of *Aloe vera*and these

results are consistent with the findings ofRoy and Sarkar (1991) and Agarwal and Barna (2004). Shoots in the initial regeneration media containing BAP alone or in combination with NAA did not produce roots. However, media containing activated charcoal irrespective of presence of citric acid induced roots after one month of culture. However, the roots initiated in these media were thin and delicate. Therefore, rooting potential in two other media, one devoid of growth hormones and other containing 0.2 mg/l NAA was evaluated. Rooting occurred within two weeks in all rooting media. 100 per cent rooting was observed in media containing 0.2 mg/l NAA with 1.3 ± 0.34 numbers of roots and 4.1 ± 0.55 cm root length, while 90 per cent rooting was observed in media containing 0.5 g/l activated charcoal irrespective of presence of citric acid and lacking hormones within two weeks of culture with 1.4 ± 0.72 numbers of root and root length was 3.1 ± 0.52 cm.

Bhandari*et al.* (2010) recorded that the rooting response in *Aloe vera* was improved in hormone free medium. The shoots inoculated on hormone free and IBA supplemented medium showed rooting response within a week. After 15 days of inoculation, rooting was 100 per cent in hormone free medium. The number of roots per shoot was 2.8 ± 0.2 on hormone free medium. In both the cases roots were without any branches and normal in appearance. Average number of roots per plant was found 2.2 ± 1.2 in medium containing hormones.

Das *et al.* (2010) obtained induction of roots in all the concentrations of *Aloe* gel without addition of sucrose and growth regulators. For induction of roots different concentrations of 2.45-9.8 µM IBA and 2.69-10.64 µM NAA were tried separately and obtained 80 per cent root induction in 2.45 µM IBA and 77 per cent root induction in 2.69 µM NAA. The highest percentage (100 per cent) of rooting with 10.90 ± 0.17 numbers of root and 3.02 ± 0.11 root lengths was obtained whileusing*Aloe* gel in rooting medium.

Biswas*et al.* (2013) observed that rooting percentage was improved in presence of low concentrations of IBA and NAA and do not support 100 per cent rooting in *Aloe vera*in hormone free medium. No adventitious roots were initiated in auxin free media. Old leaves and shoots greater than 10 cm in size did not induce adventitious roots under any conditions. NAA (0.5 mg/l) was most effective for bringing about improvements in induction rate (90 per cent), 5.2 numbers of adventitious roots per explants during six weeks of culture.

Dwivedi*et al.* (2014) reported highest percentage of root induction (80 per cent) in *Aloe vera* on MS medium supplemented with 0.5 mg/l IBA. Healthy roots i.e. more than 10 numbers of roots having root length of more than 6 cm was obtained in the same medium after 8 weeks of culture.

Gupta *et al.* (2014) found auxins (NAA) as best for root induction. Root formation was induced in *in vitro* regenerated shoots by culturing them on half strength of MS medium supplemented with 0.5 to 1.5 mg/l of any of the three plant growth regulators IBA, NAA or IAA. Root formation was not observed when shoots were cultured on a mediumlacking auxin. It was also reported that the highest root multiplication in *Aloe vera*was found in MS medium containing BAP 1.0 mg/l and IBA 0.2 mg/l. 90 per cent of root formation took place and the maximum number of root and shoot produced was 4.8 ± 0.53 with average length of 3.5 ± 0.35cm.

Khanamand Sharma(2014) observed root induction in *Aloe vera* on MS medium supplemented with 2.0 mg/l IBA and 1.0 mg/l NAA in combination. Within two weeks maximum roots per explants on this combination was 3.4 ± 0.47 and length of roots were found 3.9 ± 0.62 cm and after 4 weeks number of maximum roots per explants was 6.7 ± 0.31 and length of roots were found 7.1 ± 0.53. The least number of roots per explants was zero found in hormone free medium.

Kiran*et al.* (2017) reported rooting in newly formed shoots on rooting media in *Aloe barbadensis*. For rooting four types of growth regulators MS (half) + 0.5 mg/l IBA + 500mg/l activated charcoal, MS (half) + 0.5 mg/l IBA, MS (half) + 0.5 mg/l IAA + 500mg/l activated charcoal, basal MS (half) medium +500 mg/l activated charcoal were used in different concentration in addition to MS medium and observation was recorded for rooting response. Basal MS medium devoid of phytohormones also induced rooting but percentage of rooting and number of roots per shoot were low. IBA was found best for induction of roots followed by IAA. IBA supplemented at 1.0 mg/l induced highest frequency of rooting. The best result was obtained by using MS medium with 1.0 mg/l IBA + 500 mg/l activated charcoal.

2.5 ACCLIMATIZATION OF *IN VITRO* PRODUCED PLANTLETS

The plantlets obtained through micropropagation should have roots that are capable of supporting further growth and development. They are usually transplanted into hardening media and kept in partial shade at a high ambient humidity for several days.The term media is sometimes used to describe the mixture of materials such as peat, perlite, vermiculite, rockwool, sand and soil used for transferring the plantlets from *in vitro* conditions. Compost which is commonly used for rooting conventional cuttings is suitable for transferring these plantlets, but there may be marked differences in root growth and plantlet survival with different media.

Hardening and *ex vitro* establishment of micropropagated plants in *Aloe vera*was achieved with appreciable success by most of the investigators and high percentage of whole plant recovery was reported (Abrie and Staden, 2001, Liao *et al.,* 2004, Ahmed *et al.,* 2007, Hashemabadi and Kaviani, 2008, Singh *et al.,* 2009, Bhandari*et*

al., 2010, Das *et al.,* 2010, Nayanakantha*et al.,* 2010, Kumar *et al.,* 2011, Biswas*et al.,* 2013 and Gupta *et al.,* 2014).

Rooted plantlets were potted in a mixture of potting soil, vermiculite and sand (1:1:1) and acclimatized in a mist house (Liao *et al.,* 2004). Young Chinese aloes were planted in the field very successfully (93 per cent).

Ahmed *et al.*(2007) recorded 82 per cent of survivability in mixture of garden soil, compost and sand in a proportion of 2:1:1, where rapid shoot length was also observed. It was also revealed that regenerated plants were morphologically similar to the mother (control) plant.

Hashemabadi and Kaviani (2008) successfully acclimatized the plantlets in plastic pots containing a mixture of cocopeat and perlite (1:1) covered with transparent plastic. The result of acclimatization showed that 95 per cent of plantlets survived to grow under greenhouse conditions and were morphologically similar to mother plants.

Singh *et al.* (2009) found *ex vitro* plants to be easier to harden than *in vitro* plants. When *ex vitro* rooting of shoots and hardening of plantlets are achieved in the greenhouse in a single step, the protocol takes a shorter time for plantlet production and is several times cheaper.

Bhandari*et al.* (2010)transplanted rooted plantlets of *Aloe vera*to plastic pots containing garden soil and farmyard manure (1:1) for their hardening. Among the 90 per cent survival plants, some plants showed the symptoms of leaf tip necrosis during shade house condition.

Nayanakantha*et al.* (2010) successfully obtained well developed rooted plantlets of *Aloe vera* (4.5-5.0 cm long) after two months of

culture and 100 per cent of explants survived during and after acclimatization in pots in the plant house.

Kumar *et al.* (2011) reported transfer of rooted plantlets of *Aloe vera* from culture bottles to plastic cups in mixture of 1:1 ratio of soil and FYM for their hardening prior to their final transfer to the field showed good percentage of survival (85 per cent) in both polyhouse and shade house. In shade house, plantlets showed 82 per cent survival rate.

Bonyanpour and Khui (2013) cultured leaf explants of a local cultivar of dwarf pomegranate on MS medium supplemented with various concentrations of BAP and NAA for callus induction. Highest callus proliferation observed on a medium containing 1.0 mg/l BAP and 1.0 mg/l NAA. The highest number of shoots (7 shoots per explant) was obtained by transferring the calli to the media containing 5.0 mg/l BAP with 0.1 mg/l NAA. Maximum shoot proliferation was observed when shoots were cultured on WPM supplemented with 5.0 mg/l Kn and reported root induction on *in vitro* developed shoots on WPM medium containing IBA with 100 per cent roots on cultured shoots. Rooted plantlets were shifted in a soil mixture containing vermiculite (60 per cent), perlite (30 per cent) and coco peat (10 per cent) v/v. After 2 months, 80 per cent of plants survived and transferred to the greenhouse.

Gupta *et al.* (2014) carried out hardening of rooted plants in pots containing 1:2 (v/v) mixture of sterile sand and soil in the greenhouse at 25 ± 2ºC under 2000 lux light intensity provided by white fluorescent lamps for 16 hours photoperiod. During the first week of hardening period, regenerated plants were covered by perforated polythene sheets for maintaining high humidity and irrigated with sterile distilled water, followed by irrigation with tap water in the second week. After an additional 2-3 weeks of incubation hardened plants were transferred to

the field. Plants which were transferred directly to the field did not survive. The plantlets with well developed roots were transferred to polythene bags and the acclimatized plants were finally transferred to soil with 70 per cent survival rate.

Parmar*et al.* (2015) observed that half strength MS medium containing 0.1 mg/l NAA and 0.02 per cent activated charcoal was most effective for rooting of shoots resulting into 80.12 per cent root regeneration frequency. Regenerated plantlets were transferred to plastic cups containing autoclaved peat moss and successfully acclimatized with85 per cent survival rate. This is the first report on *in vitro* plant regeneration through direct organogenesis in *P. granatum*L. cv. Kandhari Kabuli using cotyledon explants.

Kiran *et al.* (2017) reported that regenerated plantlets of *Aloe vera* of 6-8 weeks old with well developed roots when removed from culture vessels, washed thoroughly with the tap water to remove the Agar media, the roots were treated with 0.2 per cent bavistin for 30 to 45 seconds and transferred to sterilized cocopeat in poly house and after 1 week, plants were transferred to sterilized earthen pots containing a mixture of sand, FYM, soil at a ratio of 1:2:1 and finally the potted plants were kept in the net house for acclimatization before transfer to the open field, watered at two days interval, more than 85 per cent of the potted plants survived after one month of transfer and could be successfully transferred to the field.

Surafel *et al.* (2018) reported that during rooting and acclimatization the selected treatment yielded 100 per cent rooted plants and 92.59 survival percentage in *Aloe vera* during acclimatization on 3:1 mixture of filter cake and sand after their fifth week. Application of this protocol helps to propagate thousands of *Aloe vera*plants from few initial mother plants in a year period.

Chapter-3

MATERIAL AND METHODS

The present investigation entitled “Influence of Plant Growth Regulators, Genotypes and Explants on Micropropagation of Gwarpatha [*Aloe vera*(L.) Burm.]”was carried out in Tissue Culture Laboratory, Department of Plant Breeding and Genetics, S. K. N. College of Agriculture, Jobner. Different experiments were conducted in order to achieve the objectives of the present investigation. The details of material and methods followed during the course of investigation are presented in this chapter under separate headings:

3.1 MATERIAL

3.1.1 Plant material

Present investigation was conducted on three genotypes of *Aloe vera*namely, Jobner*Aloe-1,* Jobner*Aloe-2 and* Jobner*Aloe-*3. The characteristics and photographs of these genotypes are presented in Table 3.1 and Fig. 3.1. These were obtained from nursery of *Aloe vera*accessions beingmaintained at Department of Plant Breeding and Genetics, S.K.N. College of Agriculture, Jobner.Two explants *viz.,*apical shoots and auxiliary shoots were used as explants in the present investigation.

3.1. 2 Chemicals

All chemicals used in the present study were of analytical grade. Murashige and Skoog (1962) medium was used throughout the course of investigation. The chemical composition of MS medium is given in Table 3.2.

3.1.3 Glassware

All glasswaresuchas Erlenmeyer flasks (conical flasks),culture testtubes, round bottom flask, flat bottomflasks, pipettes, petridishes, beaker, measuring cylinders (50 ml, 100 ml, 500 ml,1000 ml and 2000 ml)andambercolouredbottles (usedtostorephotosensitivesolutions) were used for media preparation.

3.2 METHODS

3.2.1 Preparation of stock solution of growth regulators

Stock solutions of the constituents were prepared and stored in a refrigerator at 4^0C. To prepare a given quantity of medium, stock solutions were mixed proportionately and the final volume was made after dissolving the sugar, agar and additives. Stock solutions of plant growth regulators were prepared. The auxins (IBA and NAA) were first dissolved in a few drops of absolute alcohol whereas cytokinin (BAP) was dissolved in a few drops of N/10 NaOH and the final volumes were made up by adding distilled water. Stock solutions of growth regulators were stored in a refrigerator and used within two weeks of its preparation. For all the purposes, double glass distilled water was used. The pH of the medium was adjusted at 5.8 using 1N NaOH or 1N HCl solution before autoclaving. Plant growth regulators were added to the basal medium before autoclaving.

3.2.2 Sterilization of glassware and culture media

Glassware were first washed with Rankleen biodegradable detergent and then washed thoroughly with tap water to remove detergent completely. Finally they were rinsed with distilled water and sterilized in oven at 160-180^0C for 3-4 hours. All the glass wares in use were regularly cleaned to ensure no contamination. Small equipments used for inoculation and explants preparation devices viz., scalpels, forceps, spatula, needles, holder were made of stainless steel and were sterilized by flame sterilization every time before use.

The culture media contained in glass containers or phyta jars sealed with non-absorbent cotton plugs and covered with aluminum foils or caps wereautoclaved at 15 psi and 121^0C for 15- 40 minutes. Exposure time depends on the volume of the liquid to be sterilized. Utmost care was taken. After autoclaving the media were stored in dark for 48 hours at 25 $\pm$ 2^0C. Thensterilizedmediumwaskept at room temperature for the purpose of storing. The prepared media was used within one week.

3.3 PREPARATION OF TRANSFER AREA FOR ASEPTIC CULTURE

The maintenance of aseptic conditions is essential for successful *in vitro* propagation. Aseptic culture works like final surface sterilization of plant material. Preparation and inoculation of explants and further sub culturing of *in vitro* cultures were carried out in a laminar air flow cabinet. Initially before the use of the cabinet, the working surface was sterilized by swabbing the surface of the cabinet with 95 per cent ethyl alcohol. Later the cabinet was ensured sterile by switching on UV light for 30 minutes followed by 30 minutes dark before use. Aseptic condition of transfer area is maintained by installing HEPA filter ventilation unit. Then the sterile airflow was switched on to avoid the entry of microbes in laminar air flow cabinet during the course of transfer. After the completion of sterile transfer operation, the laminar airflow was cleaned and sprayed with 95 per cent ethyl alcohol. The culture rooms were cleaned by gently washing all the floors and walls with a detergent soap followed by daily cleansing with phenyls.

3.4 TYPES OF EXPLANTS

For micropropagation of *Aloe vera*, two types of explants were used:

1. Apical shoot: Apical shoots wereusedasexplantsafterremoving the surrounding leaves.

2. Auxiliary shoot explants:Auxiliary shootexplants wereused after removing the apical shoot.

3.5 EXPLANTS PREPARATION AND STERILIZATION

Micro shoots with young leaves were collected of all the three genotypes. The explants apical shoots and auxiliary shoots were washed thoroughly in running tap water for 10 minutes. These were again washed with liquid detergent (Rankleen, Ranbaxy, India) for ten minutes with vigorous shaking. After washing with detergent, explants were again washed with running tap water to remove any trace of detergent for 15 minutes. Explants were surface sterilized with 0.1% $HgCl_2$for 7-10 minutes in laminar air flow cabinet. These were thoroughly washed thrice with sterilized distilled water and inoculated on the culture media supplemented with plant growth regulators.

3.6 INOCULATION OF EXPLANT

After sterilization the explants were inoculated on culture media aseptically. For inoculation, explants were transferred to large sterile glass petriplates with the help of sterile forceps under strict aseptic conditions. Here the explants were further trimmed and extra outer leaves from explants were removed to make them suitable size (2-3 cm) with sterile scalpel blade. After cutting explants of suitable size, these were transferred to culture test tubes, phyta jars and borosil flasks containing MS medium supplemented with different plant growth regulators. After vertically inoculating the explants in culture phyta jars, test tubes and borosil flasks, the mouth of phyta jars, test tubes and borosil flasks were quickly flamed, test tubes and borosil flasks were closed with non adsorbent cotton plugs and phyta jars with caps.

3.7 CULTURE CONDITIONS

All the cultures were maintained in an air conditioned culture room at atemperature of 25 ± 2^0C under fluorescent light in a

14:10hours' photoperiod. The source of illumination consisted of 2.5 feet wide fluorescent tubes (40 watt) and incandescent bulb (25 watt).

3.7.1 Plant growth regulators

Different concentrations of plant growth regulators were incorporated singly and in combinations in the MS medium for direct shoot proliferation from auxiliary explants.

I. Plant growth regulators incorporated singly in the medium:BAP: 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5 and 6.0 mg/l

II. Plant growth regulators incorporated in combinations:BAP: (0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5 and 6.0 mg/l) + NAA: (0.1, 0.2, 0.3, 0.4, 0.5 and 0.6 mg/l) in all possible combinations.

III. MS basal medium without supplementation of any growth regulatorwas used as a control in all the experiments.

3.7.2 Induction of roots

Explants were inoculated for induction of roots in the medium supplemented with different concentrations of plant growth regulators added alone or in combinations.

I. Plant growth regulators incorporated singly in the mediumNAA/IBA: 0.5, 1.0, 1.5 and 2.0 mg/l.

II. Plant growth regulators incorporated in combinations:

NAA: (0.5, 1.0, 1.5 and 2.0 mg/l) + IBA: (0.5 and 2.0 mg/l)

III. MS basal medium without supplementation of any growth regulator was used as a control in all the experiments.

3.8 EFFECT OF EXPLANTS

Different explants like auxiliary buds and apical shoots were tested for direct shoot proliferation and root induction at most responsive level of plant growth regulators.

3.9 EFFECT OF GENOTYPES

Three genotypes were tested for direct shoot proliferation and root induction at most responsive level of plant growth regulators.

3.10 HARDENING

After 40-50 days of culture on rooting media, the plantlets were shifted to polythene bags/earthen pots for their hardening prior to final transfer to soil in natural conditions. For hardening of plants, plantlets with newly formed roots were taken out from the culture bottles with the help of forceps with utmost care to prevent any damage to the newly formed roots and dipped in lukewarm water to remove any traces of solidified agar media. After removing media, these were dipped in 1% w/v solution of Bavistin to prevent any fungal infection to newly developed plants. After Bavistin treatment the plantlets were carefully planted in polythene bags/earthen pots containing 1:1 mixture of soil and vermicompost. After planting, the plants were thoroughly watered and kept in culture room for 10 days. During this period plants were watered at an interval of 2-3 days to maintain moisture in the polythene bags/earthen pots. Then the plants were shifted to shade house with indirect sunlight. In shade house plants were also watered at an interval of 2-3 days. After 50-60 days, shade house plants were finally transferred to the field.

3.11 OBSERVATIONS RECORDED

Each treatment combination was replicated 10 times and whole experiment was repeated twice to obtain unbiased results. Cultures were observed periodically and following observations were recorded:

1. Period of initiation of shoot

Mean number of days taken for shoot initiation was calculated from day of inoculation to the day explants showed shoot initiation.

2. Number of shoots per explant

Number of shoots per explant was taken as averagenumber of shoots of all explants cultured onmultiplication media after 6 weeks and 8 weeks of inoculation.

3. Morphogenetic response (Percent)

$$\text{Per cent Morphogenetic Response} = \frac{\text{Number of explants response}}{\text{Total number of inoculated explants}} \times 100$$

4. Shoots length (cm)

It was measured by scale and taken as average length of all explants after 6 weeks and 8 weeks of inoculation.

5. Number of days taken for root induction

It was calculated from inoculation on rooting media up to appearance of first roots.

6. Number of roots

It was taken as average number of roots per shoot cultured on rooting media after 40 days of inoculation.

7. Root length (cm)

The root length of the plantlet was recorded by measuring the longest root of the plantlet after 40 days of inoculation.

8. Mean days taken for callus initiation

The mean number of days taken for the initiation of callus*i.e.* formation of undifferentiated tissues in the cultured vessels.

9. Colour and texture of the callus

Colour and texture of the callus were recorded in different treatments*i.e.* green, light green, whitish green, brown, light

brown, dark brown, yellow, pale yellow, white colour and compact, semi compact, loose, friable texture etc.

3.12 STATISTICAL ANALYSIS

Eachexperiment was conducted in completely randomized design and data were analyzed for means and standard error accordingly as described by Snedecor and Cochran (1972). Standard error wascalculated only after value transformation for the characters where response was less than 100 per cent. The value for each replication was transformed by square root transformation as follows

$\sqrt{Y + ½}$Where, Y= original value

Tests of significance were done according to Duncan's Multiple Range Test (DMRT) for different traits (Gomez and Gomez, 1984).

Table 3.1 Characteristics of *Aloe vera* genotypes

S No.	Plant characters	Genotypes		
		JA-1	JA-2	JA-3
1.	Colour	Medium green	Dark green	Dark green with golden yellow spines
2.	Plant height	2-4 feet	2-3 feet	1.5-2.5 feet
3.	Leaf width at base	4-8 cm	4-6 cm	3-5 cm
4.	Leaf weight at the age of 16-18 months	400-800 gm	300-500 gm	100-200 gm
5.	Gel recovery	25-35%	25-30%	20-25%
6.	Place of collection	Kolayat, Bikaner	Sambhar, Jaipur	Sisarma, Udaipur

Table 3.2 Constitution of Murashige and Skoog (1962) Medium

Constituents	Concentration in medium (mg/l)
NH_4NO_3	1650.000
KNO_3	1900.000
H_3BO_3	6.200
KH_2PO_4	170.000
KI	0.830
$Na_2M_0O_4$. $2H_2O$	0.250
$COCl_2$. $2H_2O$	0.025
$CaCl_2$. $2H_2O$	440.000
$MgSO_4$. $7H_2O$	370.000
$MnSO_4$. $7H_2O$	22.300
$ZnSO_4$. $7H_2O$	8.600
$CuSO_4$. $5H_2O$	0.025
Na_2 EDTA	37.250
$FeSO_4$. $7H_2O$	27.850
Thiamine HCl (vit.B_1)	0.100
Nicotinic acid	0.500
Pyridoxine HCl (vit.B_1)	0.500
Glycine	2.000
Mesoinositol	100.000
Sucrose	30000.000
Agar	8000.000

Fig. 3.1: Genotypes of *Aloe vera*

Chapter-4

RESULTS

4.1 ESTABLISHMENT OF EXPLANTS

Different explants were obtained from nursery of *Aloe vera* accessions beingmaintained at the Department of Plant Breeding and Genetics, S. K. N. College of Agriculture, Jobner. Young plants were taken from field, irrigated well during previous week. Each explant was prepared and incubated as per the procedure described in the chapter of material and methods of this manuscript and all the cultures were incubated at 25 ± 2ºC under fluorescent light in a 14:10 hours photoperiod regimes. When explants incubated on basal MS medium without any plant growth regulator, the explants did not proliferate into multiple shoots. However, they grew initially into shoots and then roots emerged after 30-35 days of incubation under *in vitro* conditions.

4.2 EFFECT OF PLANT GROWTH REGULATORS ON SHOOT PROLIFERATION

4.2.1 Effect of cytokinin (BAP) added singly in medium

When BAP was supplemented singly in MS medium, the shoot bud induction from explants was observed at all the levels (0.5-6.0 mg/l). The earliest shoot initiation was observed at 5.0 mg/l (11.5 days) followed by at 4.0 mg/l BAP (12.2 days) and the last at 0.5 mg/l. Maximum number of shoot buds induction (12.8) was observed at 4.0 mg/l BAP level followed by 6.0 mg/l level (11.2) at the end of 8 weeks of inoculation (Fig. 4.1). While lowest shoot bud induction was observed from auxiliary explants at 0.5 mg/l (3.8). The frequency of shoot proliferation was 100 per cent at all levels of BAP (0.5-6.0 mg/l). Significant differences were observed for shoot proliferation from auxiliary explants at different levels of BAP (Table 4.1).

Table 4.1: Morphogenetic effect of various concentrations of cytokinin (BAP) added singly in the MS medium on auxiliary explant of *Aloe vera*

S. No.	BAP concentration (mg/l)	Days taken in shoot initiation	Morphogenetic response (per cent)	Number of shoot buds induction/explant		Shoot length (cm)	Days to callus initiation	Colour of callus	Texture of callus
				6 weeks after inoculation	8 weeks after inoculation				
1.	0.5	18.1	100	2.1 ± 0.18 **f**	3.8 ± 0.25 **g**	3.32 ± 0.36 **e**	-	-	-
2.	1.0	16.1	100	2.2 ± 0.25 **f**	4.1 ± 0.28 **fg**	4.55 ± 0.38 **d**	-	-	-
3.	1.5	14.6	100	2.6 ± 0.16 **f**	4.4 ± 0.16 **fg**	5.33 ± 0.36 **d**	22.1 [40]	Yellowish green	Loose
4.	2.0	15.3	100	3.4 ± 0.16 **e**	5.2 ± 0.33 **ef**	6.68 ± 0.40 **c**	24.6 [20]	Yellowish green	Loose
5.	2.5	15.9	100	3.6 ± 0.27 **e**	6.1 ± 0.28 **de**	8.3 ± 0.40 **b**	28.5 [10]	Light green	Semi compact
6.	3.0	15.3	100	3.8 ± 0.25 **e**	6.8 ± 0.25 **d**	8.55 ± 0.38 **b**	-	-	-
7.	3.5	14.2	100	5.2 ± 0.25 **d**	8.4 ± 0.45 **c**	8.9 ± 0.42 **b**	-	-	-
8.	4.0	12.2	100	8.4 ± 0.16 **a**	12.8 ± 0.39 **a**	11.2 ± 0.37 **a**	-	-	-
9.	4.5	14.4	100	6.2 ± 0.25 **c**	9.2 ± 0.44 **c**	4.92 ± 0.37 **d**	-	-	-
10.	5.0	11.5	100	6.3 ± 0.26 **c**	8.4 ± 0.45 **c**	2.05 ± 0.27 **f**	-	-	-
11.	5.5	12.5	100	7.1 ± 0.18 **b**	10.4 ± 0.34 **b**	1.62 ± 0.14 **f**	-	-	-
12.	6.0	13.2	100	6.2 ± 0.25 **c**	11.2 ± 0.77 **b**	1.47 ± 0.14 **f**	-	-	-

Values followed by same letters in each column are not significantly different ($p<0.05$) using DMRT

[ ] = Value in parenthesis represents percentage of response (-) = No Response

Fig. 4.1: Shoot bud induction in *Aloe vera* in MS medium supplemented with 4.0 mg/l BAP

Fig. 4.2: Callus induction in auxiliary explant on MS medium supplemented with 1.5 mg/l BAP

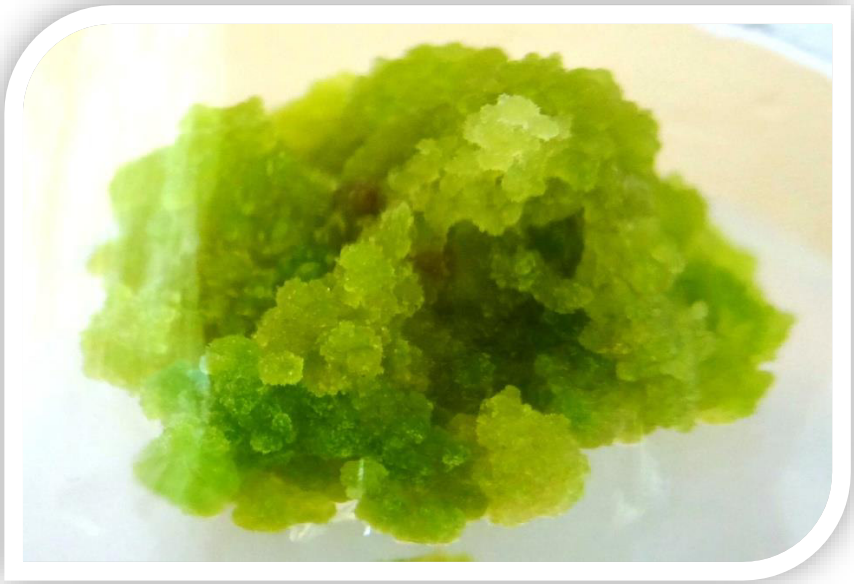

Fig. 4.3: Subculture of callus proliferated at 1.5 mg/l BAP

Maximum length (11.2 cm) of proliferated shoots was observed at 4.0 mg/l followed by 8.9 cm at 3.5 mg/l BAP level. However, increasing concentration of BAP (> 4.0 mg/l) reduced the length of shoot buds progressively and the shortest size (1.47 cm) of shootswas obtained at 6.0 mg/l BAP level. Significant differences were observed for shoot length at different levels of BAP.

After 20-25 days of incubation, slight to medium loose and semi compact callus was also induced at the base of auxiliary explants incubated at different levels of BAP (1.5-2.5 mg/l). Yellowish green callusing was proliferated from the base of explants at 1.5 mg/l BAP with 40 per cent frequency (Fig. 4.2). Lower (0.5-1.0 mg /l) and higher (3.0-6.0) levels of BAP inhibited callus differentiation. When yellowish green callus differentiated at 1.5 mg/l BAP placed on repeated subculture at various levels of BAP, this callus did not show any morphogenesis except further proliferation (Fig. 4.3). Microscopic examination of this callus revealed loose parenchymatous cells only.

4.2.2 Effect of cytokinin (BAP) and auxin (NAA) added in combinations in medium

When auxiliary explants inoculated on MS basal medium with different combinations of cytokinin (BAP) and auxin (NAA), it started to show signs of proliferation within one week. New buds started to appear from the base of explants and shoot buds developed within 2-5 weeks of culture.

Supplementationof MS medium with BAP (0.5-6.0 mg/l) and NAA (0.1-0.6 mg/l) resulted in bud break at all the levels of combinations. Maximum number of shoot bud induction (12.6) was observed at 4.5 mg/l BAP + 0.6 mg/l NAA level followed by 5.5 mg/l BAP + 0.3 mg/l NAA level (12.3) at the end of 8 weeks of inoculation (Table 4.2 and Fig. 4.4). Number of shoot bud induction was increased (20-125 per cent) at different combinations of BAP and NAA in 6 weeks

Table 4.2: Morphogenetic effect of various concentrations of cytokinin (BAP) and auxin (NAA) added in combinations in the MS medium on auxiliary explant of *Aloe vera*

S. no.	Concentration (mg/l) (BAP + NAA)	Days taken in shoot initiation	Morphogenetic response (per cent)	Number of shoot budss/explant		Shoot length (cm)
				6 weeks after inoculation	8 weeks after inoculation	
1.	0.5 + 0.1	18.2	100	2.2 ± 0.25 **x**	3.8 ± 0.249 **ab**	3.15 ± 0.33 **qrstuv**
2.	1.0 + 0.1	17.1	100	2.3 ± 0.21 **wx**	4.2 ± 0.249 **aaab**	3.31 ± 0.33 **pqrstu**
3.	1.5 + 0.1	16.2	100	2.6 ± 0.16 **vwx**	4.4 ± 0.163 **zaaab**	4.09 ± 0.39 **mnopqr**
4.	2.0 + 0.1	15.3	100	4.2 ± 0.25 **pqrs**	5.2 ± 0.2 **xyzaa**	5.24 ± 0.43 **jklm**
5.	2.5 + 0.1	15.8	100	4.3 ± 0.21 **opqrs**	6.4 ± 0.34 **stuvw**	6.99 ± 0.22 **efgh**
6.	3.0 + 0.1	15.2	100	4.4 ± 0.34 **nopqr**	6.6 ± 0.221 **rstuv**	7.5 ± 0.41 **def**
7.	3.5 +0.1	13.8	100	4.6 ± 0.27 **mnopq**	7.6 ± 0.34 **opqr**	7.95 ± 0.43 **cdef**
8.	4.0 + 0.1	12.4	100	5.4 ± 0.27 **klm**	8.8 ± 0.327 **jklm**	9.16 ± 0.43 **a**
9.	4.5 + 0.1	12.1	100	6.4 ± 0.22 **hij**	9.6 ± 0.427 **ghijk**	4.93 ± 0.37 **jklmn**
10.	5.0 + 0.1	11.3	100	7.2 ± 0.2 **efgh**	10.2 ± 0.416 **fghi**	1.95 ± 0.23 **vwx**
11.	5.5 + 0.1	12.3	100	7.2 ± 0.29 **efgh**	9.2 ± 0.359 **ijklm**	1.67 ± 0.18 **wx**
12.	6.0 + 0.1	13.2	100	8.4 ± 0.31 **cd**	10.4 ± 0.221 **fgh**	1.58 ± 0.14 **x**
13.	0.5 + 0.2	18.4	100	3.2 ± 0.25 **tuvw**	5.2 ± 0.249 **xyzaa**	2.69 ± 0.26 **stuvwx**
14.	1.0 + 0.2	16.9	100	2.4 ± 0.16 **wx**	5.6 ± 0.267 **vwxy**	3.48 ± 0.29 **opqrst**
15.	1.5 + 0.2	15.9	100	3.2 ± 0.25 **tuvw**	6.2 ± 0.327 **tuvwx**	4.26 ± 0.39 **mnopq**
16.	2.0 + 0.2	15.1	100	3.2 ± 0.25 **tuvw**	7.2 ± 0.2 **qrst**	5.74 ± 0.38 **ijkl**
17.	2.5 + 0.2	14.6	100	3.6 ± 0.27 **rstu**	7.4 ± 0.221 **pqrs**	7.0 ± 0.26 **efgh**
18.	3.0 + 0.2	13.9	100	4.2 ± 0.2 **pqrs**	7.8 ± 0.327 **nopq**	7.84 ± 0.42 **cdef**
19.	3.5 + 0.2	13.3	100	5.2 ± 0.2 **lmno**	7.2 ± 0.359 **qrst**	7.58 ± 0.36 **def**
20.	4.0 + 0.2	12.9	100	6.2 ± 0.25 **ijk**	8.6 ± 0.34 **klmn**	9.83 ± 0.45 **ab**
21.	4.5 + 0.2	11.9	100	6.6 ± 0.36 **ghij**	9.2 ± 0.389 **ijklm**	5.72 ± 0.38 **ijkl**

22.	5.0 + 0.2	11.1	100	7.2 ± 0.2 **efgh**	9.4 ± 0.306 **hijkl**	2.39 ± 0.24 **yuvwx**
23.	5.5 + 0.2	13.3	100	8.4 ± 0.4 **cd**	10.2 ± 0.249 **fghi**	1.92 ± 0.24 **vwx**
24.	6.0 + 0.2	13.1	100	9.8 ± 0.44 **a**	12.2 ± 0.291 **e**	1.57 ± 0.16 **x**
25.	0.5 + 0.3	18.6	100	2.4 ± 0.16 **wx**	5.4 ± 0.163 **wxyz**	2.97 ± 0.36 **qrstuvw**
26.	1.0 + 0.3	17.4	100	2.6 ± 0.16 **vwx**	5.8 ± 0.249 **uvwxy**	2.85 ± 0.30 **rstuvw**
27.	1.5 + 0.3	16.5	100	3.4 ± 0.27 **stuv**	6.4 ± 0.163 **stuvw**	3.41 ± 0.34 **opqrst**
28.	2.0 + 0.3	15.7	100	3.6 ± 0.27 **rstu**	6.8 ± 0.249 **qrstu**	5.73 ± 0.48 **ijkl**
29.	2.5 + 0.3	14.1	100	4.6 ± 0.16 **mnopq**	6.4 ± 0.267 **stuvw**	5.89 ± 0.64 **hijk**
30.	3.0 + 0.3	14.3	100	5.2 ± 0.25 **lmno**	8.4 ± 0.221 **lmno**	6.85 ± 0.44 **fghi**
31.	3.5 + 0.3	13.5	100	5.3 ± 0.34 **lmn**	9.6 ± 0.371 **ghijk**	7.05 ± 0.26 **efgh**
32.	4.0 + 0.3	12.5	100	6.2 ± 0.33 **ijk**	10.6 ± 0.306 **fg**	7.74 ± 0.36 **cdef**
33.	4.5 + 0.3	11.6	100	6.4 ± 0.43 **hij**	10.8 ± 0.416 **f**	5.64 ± 0.48 **ijkl**
34.	5.0 + 0.3	11.7	100	7.4 ± 0.37 **efg**	12.2 ± 0.467 **e**	3.0 ± 0.38 **qrstuvw**
35.	5.5 + 0.3	12.7	100	8.4 ± 0.27 **cd**	12.3 ± 0.359 a**b**	2.37 ± 0.29 **tuvwx**
36.	6.0 + 0.3	11.9	100	9.8 ± 0.29 **a**	12.1 ± 0.476 **e**	1.53 ± 0.14 **x**
37.	0.5 + 0.3	18.1	100	2.2 ± 0.25 **x**	5.1 ± 0.233 **yzaa**	2.99 ± 0.36 **qrstuvw**
38.	1.0 + 0.4	17.2	100	2.4 ± 0.16 **wx**	5.2 ± 0.249 **xyzaa**	3.75 ± 0.37 **nopqrs**
39.	1.5 + 0.4	16.1	100	3.4 ± 0.27**stuv**	5.2 ± 0.2 **xyzaa**	4.68 ± 0.19 **klmno**
40.	2.0 + 0.4	15.2	100	3.6 ± 0.27 **rstu**	5.6 ± 0.306 **vwxy**	5.7 ± 0.31 **ijkl**
41.	2.5 + 0.4	14.9	100	4.6 ± 0.16 **mnopq**	6.8 ± 0.249 **qrstu**	7.76 ± 0.48 **cdef**
42.	3.0 + 0.4	14.5	100	5.2 ± 0.25 **lmno**	8.2 ± 0.359 **mnop**	7.57 ± 0.41 **def**
43.	3.5 + 0.4	12.7	100	5.2 ± 0.25 **lmno**	9.2 ± 0.249 **ijklm**	7.81 ± 0.53 **cdef**
44.	4.0 + 0.4	13.1	100	6.8 ± 0.33 **fghi**	9.8 ± 0.389 **fghij**	8.04 ± 0.61 **cdef**
45.	4.5 + 0.4	11.5	100	6.4 ± 0.27 **hij**	10.1 ± 0.482 **fghi**	4.27 ± 0.34 **mnopq**
46.	5.0 + 0.4	11.2	100	7.4 ± 0.34 **efg**	10.8 ± 0.442 **f**	2.96 ± 0.33 **qrstuvw**
47.	5.5 + 0.4	12.9	100	8.4 ± 0.45 **cd**	10.8 ± 0.291 **f**	2.21 ± 0.30 **tuvwx**
48.	6.0 + 0.4	13.7	100	8.5 ± 0.43 **ab**	11.6 ± 0.163 **bc**	2.04 ± 0.27 **uvwx**
49.	0.5 + 0.5	17.9	100	2.4 ± 0.27 **wx**	5.2 ± 0.249 **xyzaa**	2.26 ± 0.22 **tuvwx**
50.	1.0 + 0.5	17.6	100	2.4 ± 0.16 **wx**	5.6 ± 0.163 **vwxy**	3.74 ± 0.37 **nopqrs**

51.	1.5 + 0.5	16.7	100	3.6 ± 0.27 **rstu**	6.6 ± 0.163 **rstuv**	4.47 ± 0.39 **lmnop**
52.	2.0 + 0.5	15.5	100	4.2 ± 0.25 **pqrs**	6.2 ± 0.2 **tuvwx**	5.27 ± 0.35 **jklm**
53.	2.5 + 0.5	14.2	100	5.2 ± 0.25 **lmno**	7.1 ± 0.277 **qrst**	6.05 ± 0.5 **ghij**
54.	3.0 + 0.5	13.7	100	5.4 ± 0.16 **klm**	7.4 ± 0.306 **pqrs**	8.24 ± 0.50 **cde**
55.	3.5 + 0.5	13.6	100	6.4 ± 0.27 **hij**	8.4 ± 0.371 **lmno**	7.87 ± 0.35 **cdef**
56.	4.0 + 0.5	11.7	100	7.6 ± 0.16 **def**	9.4 ± 0.371 **hijkl**	7.94 ± 0.38 **cdef**
57.	4.5 + 0.5	12.5	100	7.6 ± 0.27 **def**	9.8 ± 0.327 **fghij**	9.77 ± 0.63 **ab**
58.	5.0 + 0.5	11.2	100	7.4 ± 0.27 **efg**	10.6 ± 0.34 **fg**	4.81 ± 0.35 **jklmn**
59.	5.5 + 0.5	12.7	100	8.1 ± 0.28 **cde**	12.2 ± 0.163 **cd**	2.28 ± 0.23 **tuvwx**
60.	6.0 + 0.5	13.5	100	9.6 ± 0.37 **ab**	12.0 ± 0.221 **c**	1.89 ± 0.19 **vwx**
61.	0.5 + 0.6	18.7	100	2.8 ± 0.25 **uvwx**	4.8 ±0.133 **yzaa**	3.09 ± 0.32 **qrstuv**
62.	1.0 + 0.6	17.3	100	3.2 ± 0.13 **tuvw**	5.4 ± 0.163 **wxyz**	4.03 ± 0.36 **mnopqr**
63.	1.5 + 0.6	16.4	100	4.2 ± 0.25 **pqrs**	5.8 ± 0.327 **uvwxy**	4.99 ± 0.38 **jklmn**
64.	2.0 + 0.6	15.6	100	3.8 ± 0.25 **qrst**	5.1 ± 0.233**yzaa**	7.16 ± 0.31 **efg**
65.	2.5 + 0.6	14.7	100	5.1 ± 0.31 **lmnop**	6.2 ± 0.2 **tuvwx**	7.6 ± 0.42 **def**
66.	3.0 + 0.6	14.4	100	4.8 ± 0.29 **mnop**	6.8 ± 0.249 **qrstu**	8.74 ± 0.54 **bcd**
67.	3.5 + 0.6	13.9	100	5.8 ± 0.29 **jkl**	8.2 ± 0.359 **mnop**	8.27 ± 0.59 **cde**
68.	4.0 + 0.6	13.4	100	6.0 ± 0.26 **ijkl**	8.4 ± 0.306 **lmno**	8.91 ± 0.64 **bc**
69.	4.5 + 0.6	12.2	100	9.8 ± 0.42 **a**	12.6 ± 0.427 **a**	9.02 ± 0.65 **bc**
70.	5.0 + 0.6	14.2	100	6.6 ± 0.4 **ghij**	9.6 ±0.371 **ghijk**	6.76 ± 0.37 **fghi**
71.	5.5 + 0.6	13.7	100	8.4 ± 0.43 **cd**	10.4 ± 0.267 **fgh**	2.64 ± 0.29 **stuvwx**
72.	6.0 + 0.6	13.6	100	8.8 ± 0.33 **bc**	10.8 ± 0.327 **b**	1.9 ± 0.23 **vwx**

Values followed by same letters in each column are not significantly different ($p<0.05$) using DMRT

Fig. 4.4: Shoot bud induction in *Aloe vera* in MS medium supplemented with 4.5 mg/l BAP + 6.0 mg/l NAA

to 8 weeks of inoculation. The number of shoot bud break was low at lower levels of both the plant growth regulators which increased with increasing levels. However, these slightly decreased when medium supplemented with >4.5 mg/l BAP with NAA. Frequency of shoot bud break in auxiliary explants was 100 per cent at all the combinations. Significant differences were observed for shoot bud induction among different combinations of plant growth regulators after six and eight weeks of inoculation.

The earliest shoot bud initiation was observed at 0.2 mg/l NAA + 5.0 mg/l BAP level (11.1 days). Shoot bud induction was delayed (18.7 days) when explants were inoculated at 0.5 mg/l BAP + 0.6 mg/l NAA. Early bud break in explants induced higher number of shoot buds per explant in comparison to delayed shoot bud break.

At the end of culture (8 weeks), the longest shoots were obtained in the culture supplemented with 4.0 mg/l BAP + 0.2 mg/l NAA (9.83 cm) followed by 4.5 mg/l BAP + 0.5 mg/l NAA (9.77 cm) while most stunted shoots were obtained at 6.0 mg/l BAP + 0.3 mg/l NAA (1.53 cm). Shoot length was also differed significantly at different combinations of BAP and NAA. All the combinations of BAP and NAA inhibited callus induction at the base of auxiliary explants even after 8 weeks of culture.

MS medium supplemented with BAP (4.0 mg/l) alone and in combination BAP (4.5 mg/l) + NAA (0.6 mg/l) induced maximum shoot buds in auxiliary explants thus, these levels were considered as most responsive for alone and combination of plant growth regulators for bud break in *Aloe vera,* respectively.

4.3 EFFECT OF PLANT GROWTH REGULATORS ON ROOT INDUCTION

4.3.1 Effect of auxins (IBA and NAA) added singly in medium

Three to four centimeter long shoots were excised individually from the proliferated shoot clumps and cultured on rooting medium. Initially shoot bud break was observed at different levels of auxins (IBA and NAA) added singly. However, the rate of shoot bud break was very less in comparison to medium supplemented with alone or in combinations of cytokinin and auxin. The maximum shoot bud induction (2.7) was observed in the medium supplemented with 2.0 mg/l NAA. NAA induced higher number of shoots per explants in comparison to IBA supplemented medium. The shoot bud induction differed significantly between IBA and NAA, however it differed non significantly among different levels of IBA/NAA (Table 4.3).

In present investigation root induction was observed at all the levels of IBA (0.5-2.0 mg/l) and NAA (0.5-2.0 mg/l) added singly within 20-28 days of incubation with 100 per cent frequency. Maximum root induction (6.6) was observed in the medium supplemented with 1.5 mg/l IBA followed by (5.4) 1.0 mg/l IBA (Fig. 4.5). In IBA supplemented media roots were thin and long whereas, thick roots were induced in medium supplemented with NAA (Fig. 4.6). In comparison to NAA, IBA produced higher number of roots at all the levels except at 2.0 mg/l IBA. There was significant difference among the levels of IBA and NAA for number of root induction per explant.

Longest roots were obtained at 1.5 mg/l IBA (7.64 cm) followed by 0.5 mg/l NAA (5.64 cm). Shortest root induction was observed in MS medium supplemented with 2.0 mg/l IBA (1.81 cm). Significant difference was also observed for root lengths at different levels of IBA and NAA. Number of roots and root length gradually decreased with increasing level of NAA.

Table 4.3: Effect of various concentrations of auxins (IBA and NAA) added singly in the MS medium on rooting in auxiliary explants of *Aloe vera*

S. No.	Auxin concentration (mg/l)	Days taken in root initiation	Rooting response (per cent)	Number of roots/explant	Root length (cm)	Number of shoot buds/explant
1.	IBA 0.5	25.2	100	4.7 ± 0.153 **bc**	2.74 ± 0.142 **ef**	1.2 ± 0.13 **c**
2.	IBA 1.0	24	100	5.4 ± 0.163 **b**	3.92 ± 0.213 **cd**	1.2 ± 0.13 **c**
3.	IBA 1.5	21.2	100	6.6 ± 0.400 **a**	7.64 ± 0.330 **a**	1.1 ± 0.1 **c**
4.	IBA 2.0	22.4	100	2.2 ± 0.133 **e**	1.81 ± 0.142 **g**	1.3 ± 0.15 **c**
5.	NAA 0.5	26.2	100	4.6 ± 0.306 **bc**	5.64 ± 0.279 **b**	2.1 ± 0.28 **b**
6.	NAA 1.0	25.4	100	4.1 ± 0.539 **cd**	4.55 ± 0.318 **c**	2.4 ± 0.22 **ab**
7.	NAA 1.5	20	100	3.6 ± 0.340 **d**	3.24 ± 0.207 **de**	2.4 ± 0.22 **ab**
8.	NAA 2.0	20	100	3.4 ± 0.221 **d**	2.49 ± 0.291 **fg**	2.7 ± 0.15 **a**

Values followed by same letters in each column are not significantly different ($p<0.05$) using DMRT

4.3.2 Effect of auxins (IBA and NAA) added in combinationsin medium

Different auxins (IBA and NAA), when incorporated in combination in the medium did not elicit significant differences for shoot bud induction at various levels. Combination of IBA and NAA when added to MS basal medium at different levels of concentration (0.5-2.0 mg/l NAA + 0.5 and 2.0 mg/l IBA) showed 100 per cent frequency of root induction. The first root initiation was observed at 1.5 mg/l and 2.0 mg/l IBA + 2.0 mg/l NAA (10.6 days) and last at 0.5 mg/l IBA + 0.5 mg/l NAA (22.4 days). The maximum number of roots (5.6) was observed at 0.5 mg/l IBA + 2.0 mg/l NAA followed by 0.5 mg/l IBA + 1.5 mg/l NAA (Fig. 4.7) and the least number of roots observed at 2 mg/l IBA + 0.5 mg/l NAA (2.8). Longest root length was obtained at 0.5 mg/l IBA + 2.0 mg/l NAA (8.3 cm) followed by 2.0 mg/l IBA + 0.5 mg/l NAA (6.2 cm) and the shortest roots from 0.5 mg/l IBA + 0.5 mg/l NAA (3.1 cm) (Table 4.4).

Higher level of auxins (NAA and IBA) reduced number of roots induction per plant. Significant differences were observed for number of roots and root length at different combinations of auxins. MS medium supplemented with IBA (1.5 mg/l) alone and in combination IBA (0.5 mg/l) + NAA (2.0 mg/l) induced maximum roots in shoots thus, these levels were considered as most responsive for alone and combination of plant growth regulators for root induction in *Aloe vera,* respectively.

4.4 EFFECT OF EXPLANTS

In the present investigation two kinds of explant viz. apical shoot and auxiliary shoot were inoculated on media supplemented with the most responsive levels (4.0 mg/l BAP and 4.5 mg/l BAP + 0.6 mg/l NAA for shoot proliferation and 1.5 mg/l IBA and2.0 mg/l IBA + 0.5 mg/l NAA for root induction) of plant growth regulators, both the explants showed different results of shoot bud break and root induction.

Fig. 4.5: Root induction in *Aloe vera* on MS medium supplemented with 1.5 mg/l IBA

Fig. 4.6: Root induction in *Aloe vera* on MS medium supplemented with 0.5 mg/l NAA

Fig. 4.7: Root induction in *Aloe vera* on MS medium supplemented with 0.5 mg/l IBA + 2.0 mg/l NAA

Table 4.4: Effect of auxins (IBA and NAA) added in combinations in the MS medium on rooting in auxiliary explant of *Aloe vera*

S. No.	Auxin concentration (IBA + NAA) (mg/l)	Days taken in root initiation	Rooting response (per cent)	Number of roots/explant	Root length (cm)	Number of shoot buds/explant
1.	0.5+0.5	22.4	100	4.2 ± 0.291 **bcd**	3.1 ± 0.317 **d**	1.3 ± 0.15 **d**
2.	0.5+ 1.0	16.8	100	4.5 ± 0.522 **abc**	4.2 ± 0.384 **cd**	1.8 ± 0.2 **cd**
3.	0.5+1.5	14.2	100	4.8 ± 0.467 **ab**	5.6 ± 0.45 **b**	2.1 ± 0.23 **bc**
4.	0.5+2.0	13.6	100	5.8 ± 0.742 **a**	8.3 ± 0.448 **a**	2.3 ± 0.26 **ab**
5.	2.0+0.5	15.6	100	2.8± 0.327 **d**	6.2 ± 0.429 **b**	2.2 ± 0.25 **ab**
6.	2.0+1.0	12.8	100	3.9 ± 0.458 **bcd**	5.8 ± 0.404 **b**	1.8 ± 0.2 **cd**
7.	2.0+1.5	10.9	100	3.4 ± 0.340 **bcd**	5.5 ± 0.390 **b**	2.5 ± 0.22 **ab**
8.	2.0+ 2.0	10.6	100	3.1 ± 0.379 **cd**	5.0± 0.377 **bc**	2.8 ± 0.13 **a**

Values followed by same letters in each column are not significantly different ($p<0.05$) using DMRT

Table 4.5: Effect of differentexplantsof*Aloe vera*at most responsive levels of plant growth regulators (4.0 mg/l BAP and 4.5 mg/l BAP + 0.6 mg/l NAA) on shoot proliferation

S.No.	Explant	Days taken in shoot inititation	Morphogenetic response (per cent)	Number of shoot buds/explant		Shoot length (cm)	Days to callus initiation
				6 weeks	8 weeks		
4.0 mg/l BAP							
1.	Apical shoot	22.3	100	3.1 ± 0.233 **b**	5.3 ± 0.25 **b**	3.09 ± 0.19 **b**	**-**
2.	Auxiliary shoot	15.5	100	7.3 ± 0.260 **a**	11.0 ±0.39 **a**	9.65 ± 0.51 **a**	**-**
4.5 mg/l BAP+ 0.6 mg/l NAA							
1.	Apical shoot	20.5	100	3.7 ± 0.213 **b**	5.7± 0.213 **b**	4.3 ± 0.18**b**	**-**
2.	Auxiliary shoot	12.2	100	8.2 ± 0.327 **a**	12.8± 0.327**a**	8.53 ± 0.44 **a**	**-**

Values followed by same letters in each column are not significantly different ($p<0.05$) using DMRT

Fig. 4.8: Shoot proliferation in Apical shoot explants at medium supplemented with 4.0 mg/l BAP

Fig. 4.9: Shoot proliferation in Auxiliary shoot explants at medium supplemented with 4.0 mg/l BAP

Fig. 4.10: Shoot proliferation in apical shoot explants at medium supplemented with 4.5 mg/l BAP + 0.6 mg/l NAA

Fig. 4.11: Shoot proliferation in auxiliary shoot explants at medium supplemented with 4.5 mg/l BAP + 0.6 mg/l NAA

4.4.1 Effect of explants on shoot bud proliferation

Among the two kinds of explants auxiliary shoot responded considerably better on the media for shoot proliferation. Auxiliary shoot explants started proliferating at 15.5 days after inoculation while apical shoot explants started 22.3 days after inoculation. Frequency of shoot proliferation shoot was 100 per cent in both the explants. Further, after 8 weeks the auxiliary shoot explants showed higher number of shoots (11.0), while apical shoot explants resulted into lesser numbers of shoots proliferated (5.3).

The length of shoots was also higher at auxiliary explants (9.65 cm) and lower at apical explants (3.09 cm). Significant differences were observed among the explants for shoot proliferation and shoot length at same level of plant growth regulator (Table 4.5, Fig. 4.8 and Fig. 4.9).

When MS medium supplemented with 4.5 mg/l BAP + 0.6 mg/l NAA auxiliary shoot explants started proliferating at 12.2 days after inoculation while apical shoot explants started 20.5 days after inoculation. The frequency of shoot proliferation was 100 per cent in both the explants. After 8 weeks of inoculation the auxiliary shoot explants showed higher number of shoots (12.8), while apical shoot explants resulted into lesser numbers of shoots proliferated (5.7).

The length of shoots was also higher at auxiliary explants (8.53 cm) and lower at apical explants (4.3 cm). Callusing was not repoeted in both the explants supplemented with these plant growth regulator levels. Significant differences were observed for shoot length and number of shoots between both the explants at same levels of plant growth regulators (Table 4.5, Fig. 4.10 and Fig. 4.11).

4.4.2 Effect of explants on root induction

When proliferated shoots from both the explants were inoculated on media supplemented with most responsive levels of auxins, there was 100 per cent frequency of root induction for both the explants. At 1.5 mg/l IBA auxiliary explants started rooting at 22.3 days after inoculation, while apical shoot explants started at 29.8 days after inoculation. Auxiliary shoot explants induced more number of roots (6.8) than apical shoot explants (2.9). Length of the roots was more in auxiliary shoot explants (7.89 cm) while shorter roots (2.25 cm) were observed in apical shoot explants. Significant difference was found between the length and number of shoots at both the explants. (Table 4.6 and Fig. 4.12).

When proliferated shoots from both the explants were inoculated on media supplemented with most responsive levels of auxins (2.0 mg/l IBA + 0.5 mg/l NAA) auxiliary explants started rooting at 18.3 days after inoculation, while apical shoot explants started at 24.2 days after inoculation. Auxiliary shoot explants induced more number of roots (5.6) than apical shoot explants (3.7). Frequency of root induction was 100 per cent in both the explants.

Length of the roots was more in auxiliary shoot explants (8.02 cm) while shorter roots (3.17 cm) were observed in apical shoot explants (Table 4.6 and Fig. 4.13). Only few shoots were proliferated in auxiliary explants (2.3) and apical shoot explants (1.1).

On the basis of above results auxiliary explants was found more responsive for shoot bud break and root induction cultured on most responsive levels of plant growth regulators supplemented alone or in combination.

Fig. 4.12: Root induction from auxiliary and apical explants at medium supplemented with 1.5 mg/l IBA

Fig.4.13: Root induction from auxiliary and apical explants at medium supplemented with 0.5 mg/l IBA + 2.0 mg/l NAA

Table 4.6: Effect of differentexplantsof*Aloe vera*at most responsive levels of plant growth regulators (1.5 mg/l IBA and 2.0 mg/l IBA + 0.5 mg/l NAA) on root induction

S. No.	Explant	Days taken in root initiation	Rooting response (per cent)	Number of roots/explant	Root length (cm)	Number of shoot buds/explant
1.5 mg/l IBA						
1.	Apical shoot	29.8	100	2.9 ± 2.333 **b**	2.25± 0.150 **b**	1.3 ± 0.15 **b**
2.	Auxiliary shoot	22.3	100	6.8 ± 0.367 **a**	7.89± 0.224 **a**	1.9 ± 0.23 **a**
2.0 mg/l IBA + 0.5 mg/l NAA						
1.	Apical shoot	24.2	100	3.7 ± 0.197 **b**	3.17± 0.277 **b**	1.1 ± 0.1 **b**
2.	Auxiliary shoot	18.3	100	5.6 ± 0.327 **a**	8.02 ± 0.319 **a**	2.3 ± 0.26 **a**

Values followed by same letters in each column are not significantly different ($p<0.05$) using DMRT

4.5 EFFECT OF GENOTYPES

Three genotypes of *Aloe vera* namely JA-1, JA-2 and JA-3 were tested for shoot proliferation and root induction on most responsive levels of plant growth regulators.

4.5.1 Effect of genotypes on shoot proliferation

When all the genotypes were cultured on MS medium supplemented with 4.0 mg/l BAP for shoot bud break. Genotype JA-1 showed bud break within 18.7 days of inoculation with 100 per cent frequency. Genotype JA-3 remained dormant upto 25 days of inoculation and later on bud differentiation was observed at 29.6 days of inoculation. Maximum number of shoots (3.6) was observed in JA-1 followed by JA-2 (2.8) with 90 per cent frequency and least number (1.8) of shoots in JA-3 with 60 per cent frequency (Fig. 4.14, Fig. 4.15 and Fig. 4.16). Longest shoots were recorded in JA-1 (3.3 cm) followed by JA-2 (2.8 cm) and shortest shoots (1.75 cm) in JA-3 (Table. 4.7).

Further, perusal Table 4.7 revealed that significant differences were observed in different genotypes for shoot bud proliferation and shoot length at the same level of plant growth regulator.

On MS medium supplemented with 4.5 mg/l BAP + 0.6 mg/l NAA maximum number of shoots were observed in JA-1 (4.1) followed by JA-2 (3.5) and least number of shoots in JA-3 (Table 4.7 and Fig. 4.17).

Frequency of shoot proliferation was 100 per cent in JA-1 and JA-2 whereas, it was 50 per cent in JA-3. Longest shoots were recorded in JA-1 (4.06 cm) followed by JA-2 (2.16 cm) and shortest (1.31 cm) shoots in JA-3. All genotypes differed significantly for number of shoot proliferation and shoot length at same level of plant growth regulators.

Fig. 4.14: Shoot proliferation in genotype JA-1 at medium supplemented wtih 4.0 mg/l BAP

Fig. 4.15: Shoot proliferation in genotype JA-2 at medium supplemented wtih 4.0 mg/l BAP

Fig. 4.16: Shoot proliferation in genotype JA-3 at medium supplemented wtih 4.0 mg/l BAP

Fig. 4.17: Shoot proliferation in genotypes JA-1, JA-2 and JA-3 on medium supplemented with 4.5 mg/l BAP + 0.6 mg/l NAA

Table 4.7: Effect of differentgenotypesof*Aloe vera*at most responsive levels of plant growth regulators (4.0 mg/l BAP and 4.5 mg/l BAP + 0.6 mg/l NAA) on shoot proliferation

S.No.	Genotype	Days taken in shoot inititation	Morphogenetic response (per cent)	Number of Shoots/explant		Shoot length (cm)
				6 weeks after inoculation	8 weeks after inoculation	
4.0 mg/l BAP						
1.	JA-1	18.7	100	2.9* ± 0.04 **a**	3.6* ± 0.03 **a**	3.3 ± 0.04 **a**
2.	JA-2	25.3	90	2.04 ± 0.16 **b**	2.8± 0.23 **b**	1.75± 0.13 **b**
3.	JA-3	29.6	60	1.45 ± 0.21 **c**	1.8 ± 0.29 **c**	1.22± 0.14 **c**
4.5 mg/l BAP+ 0.6 mg/l NAA						
1.	JA-1	15.6	100	3.6 ± 0.05 **a**	4.1 ± 0.17**a**	4.06± 0.03 **a**
2.	JA-2	24.1	100	2.4± 0.04 **b**	3.5± 0.27 **b**	2.16± 0.03 **b**
3.	JA-3	27.9	50	1.3 ± 0.21 **c**	1.5 ± 0.2 **c**	1.31 ± 0.20 **c**

Values followed by same letters in each column are not significantly different ($p<0.05$) using DMRT

(*) = Transformed value

4.5.2 Effect of genotypes on root induction

Different results were recorded for root induction among the three genotypes of *Aloe vera.* When auxiliary explants of all the genotypes were incubated on MS medium supplemented with 1.5 mg/l IBA showed root initiation at the base of explants within 17-24 days (Fig. 4.18, Fig. 4.19 and Fig. 4.20). The highest number of roots was induced in JA-2 (7.7) followed by JA-1 (6.1) and least number in JA-3 (4.8). Frequency of root induction was 100 per cent in all the genotypes. Longest roots were observed in JA-2 (7.82 cm) followed by JA-1 (6.37 cm).

When MS media supplemented with 0.5 mg/l IBA + 2.0 mg/l NAA, the highest number of roots (9.8) was induced in JA-2 followed by (5.4) JA-1 and least number of roots (4.8) were in JA-3 (Fig.4.21). Longest roots were induced in JA-2 (8.93 cm) followed by JA-1 (7.91cm) and shortest roots in JA-3 (3.03 cm).

Further, perusal Table 4.7 revealed that significant differences were observed in all the genotypes of *Aloe vera* for root induction, root length and shoot proliferation at 0.5 mg/l IBA + 2.0 mg/l NAA.

4.5 HARDENING OF PLANTLETS

After 40-50 days of culture of *in vitro* developed shoots on most responsive rooting media (supplemented with 1.5 mg/l IBA singly and 2.0 mg/l IBA + 0.5 mg/l NAA in combination) which resulted in sufficient rooting of the shoots were shifted to pots prior to final transfer to soil in natural conditions for hardening of plants. Plants with newly developed roots were taken out from the culture bottles with the help of forceps with utmost care to prevent any damage to newly formed roots and dipped in lukewarm water to remove traces of semi solid agar media. After removing medium, plants were dipped in 1.0 percent solution of bavistin to prevent any fungal infection to newly developed plants. After bavistin treatment the plantlets were carefully planted in trays

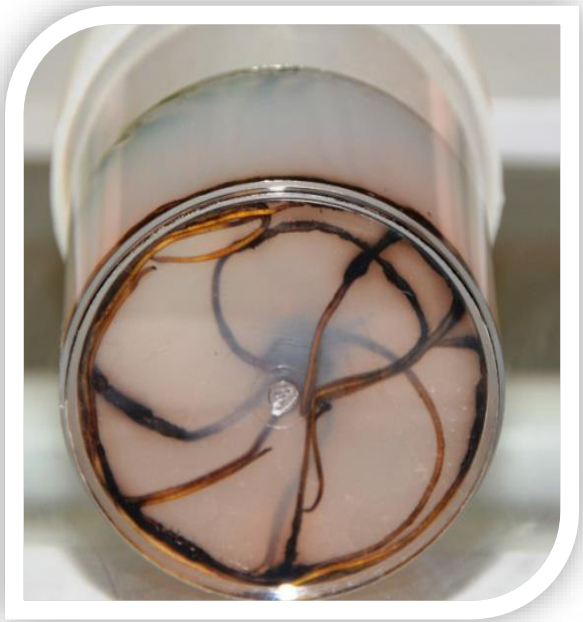

Fig. 4.18: Root induction in JA-2 on medium supplemented with 1.5 mg/l IBA

Fig. 4.19 Root induction in JA-1 on medium supplemented with 1.5 mg/l IBA

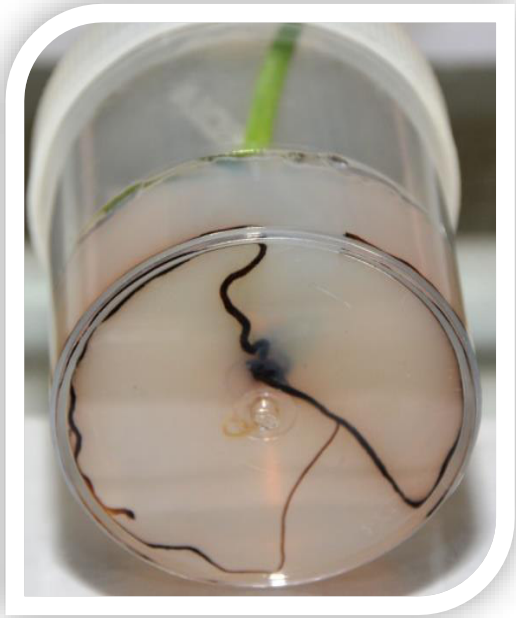

Fig. 4.20: Root induction in JA-3 on medium supplemented with 1.5 mg/l IBA

Fig. 4.21: Root induction in genotypes JA-1, JA-2 and JA-3 on medium supplemented with 0.5 mg/l IBA + 2.0 mg/l NAA

Table 4.8: Effect of differentgenotypesof*Aloe vera*at most responsive levels of plant growth regulators (1.5 mg/l IBA and 2.0 mg/l IBA + 0.5 mg/l NAA) on root proliferation

S.No.	Genotype	Days taken in root inititation	Morphogenetic response (per cent)	Number of roots/explant	Root length (cm)	Number of shoots
1.5 mg/l IBA						
1.	JA-1	22.6	100	6.1± 0.38 **b**	6.37± 0.26 **b**	2.2 ± 0.25 **a**
2.	JA-2	18.1	100	7.7± 0.21 **a**	7.82 ± 0.27 **a**	1.3 ± 0.15 **b**
3.	JA-3	20.2	100	4.8 ± 0.33 **c**	2.96 ± 0.15 **c**	1.1 ± 0.1 **c**
2.0 mg/l IBA + 0.5 mg/l NAA						
1.	JA-1	19.6	100	5.4± 0.37 **b**	7.91 ± 0.46 **b**	2.4 ± 0.22 **a**
2.	JA-2	15.3	100	9.8 ± 0.25 **a**	8.93 ± 0.29 **a**	1.5 ± 0.17 **b**
3.	JA-3	18.3	100	3.3 ± 0.26 **c**	3.03 ± 0.15 **c**	1.3 ± 0.15 **c**

Values followed by same letters in each column are not significantly different ($p<0.05$) using DMRT

containing 1:1 mixture of soil and vermicompost. After planting, the plants were thoroughly watered and kept in culture room for 10 days. During this period, plants were thoroughly watered at an interval of 2-3 days to maintain moisture in trays. Then the plants were shifted to shade house under less humidity and indirect sunlight. In shade house plants were also watered at an interval of 2-3 days. After 40 days shade house plants were finally transferred to field.

Table 4.9: Survival rate of plantlets of *Aloe vera* at different stages of hardening

Stage of Transplantation	Number of plants transplanted	Percentage of survival
Culture room (I stage)	200	80
Shade house (II stage)	160	74

Plantlets that were transferred to the trays in culture room showed good percentage (80 per cent)(Table 4.9 and Fig. 4.22).In shade house,these plantsstarted to elongate and leaves also started to thicken (Fig. 4.23). Among the survived plants some showed the symptoms of leaf tip necrosis during shade house conditions, but this did not hamper the overall growth of the plants. Plants with these symptoms were also growing well.

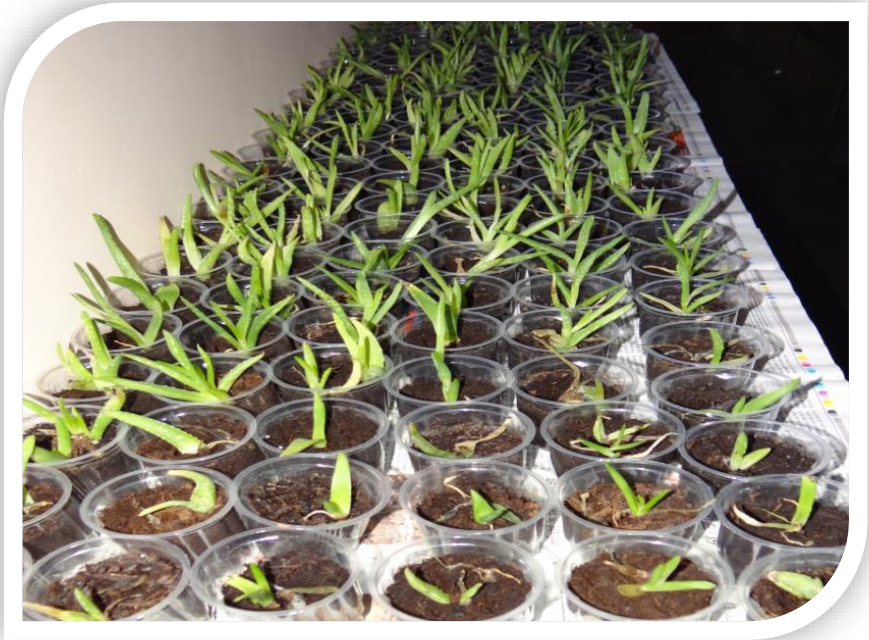

Fig. 4.22 Hardening of *in vitro* rooted *Aloe vera* plantlets

Fig. 4.23: Fully hardened plantlets of *Aloe vera* growing in polyhouse

Chapter- 5

DISCUSSION

Tissue culture is the cultivation of plant cells, tissues and organs on specially formulated nutrient media.Over 100 years ago, Haberlandt envisioned the concept of plant tissue culture and provided the groundwork for the cultivation of plant cells, tissues and organs in culture. It relies on phenomenon of cell totipotency, the latter being the ability of single cells to divide, produce all the differentiated cell characteristics of organs and regenerate into a whole plant. The technique of plant tissue culture is used for growing isolated plant cells, tissues and organs under axenic conditions (*in vitro*) to regenerate and propagate entire plants.

Tissue culture techniques include embryo culture, anther culture, induction of somaclonal variations, micropropagation, protoplast culture, cryopreservation, production of synthetic seed and production of secondary metabolites. Among these cultures micropropagation has become an important part of the commercial propagation of many plants because of its advantages as a multiplication system. Fertile plants can be regenerated either by the growth and proliferation of existing auxiliary and apical meristems or by the regeneration of adventitious shoots. Adventitious buds and shoots are formed *de novo,*meristems are initiated from explants, such as those of leaves petioles, hypocotyls, floral organs and roots. (Iliev *et al.,* 2010)

Today plant tissue culture applications encompass much more than clonal propagation. The range ofroutine technologies has expanded to include somatic embryogenesis, somatic hybridization,

viruselimination as well as the application of bioreactors to mass propagation.Plant tissue culture technique though an underutilized tool, it can be extensively applied in horticulture and agriculture to increase crop production.

Plant tissue culture is now a well-established technology.Like many other technologies, it has gone through different stages of evolution, scientific curiosity, researchtool, novel applications and mass exploitation. Initially,plant tissue culture was exploited as a research tool andfocused on attempts to culture and study the developmentof small, isolated segments of plant tissues orisolated cells. Around the mid twentieth century, thenotion that plants could be regenerated or multiplied fromeither callus or organ culture was widely accepted andpractical application in the plant propagation industryensued. The technique was heralded as the universalmass clonal plant propagation system for the future andthe term 'micropropagation' was introduced to describe more accurately the processes (Akin-Idowu *et al.,* 2009).

However, the capacity to propagate '*in vitro*' is generally influenced by genotypes of particular species, tissues of specific organs, appropriate chemical and physical environment used for culturing. Most of the researchers try to use optimum physical and physiological conditions for proper growth of cells, tissues, organs and plants, empirically manipulate ratio of plant growth regulators to affect cell proliferation and differentiation.

Demand of *Aloe vera,* has increased many fold in recent past due to its highly priced cosmetic and medicinal properties and it is being used almost in every cosmetic product these days (Cera *et al.,* 1980, Davis and Leiter,1988, Afzal *et al.,* 1991 and Reynolds and Dweck,1999). In nature, it propagates vegetatively but the rate is very

slow and is highly dependent on climatic conditions. This species is also affected by a widespread male sterility (Keijzer and Cresti, 1987). Thus the availability of quality and true to type planting material is going to be a limiting factor in *Aloe* cultivation in near future. Perusal of review reveals that micropropagation of members of *Aloe* have been influenced by the type of plant growth regulators and their levels used either singly or in combinations.

In the present investigation, attempts were made to optimize the conditions for shoot multiplication through the manipulation of plant growth regulators and assess the effect of different explants and genotypesunder *in vitro* conditions of *Aloe vera.*

While discussing the results, efforts have been made to compare and analyse these with the work already published. Since voluminous literature is available on totipotency of plant cells, it is not possible to refer all the previous interpretation. However, relevant references have been cited both from related and other genera.

5.1 EFFECT OF PLANT GROWTH REGULATORS ON SHOOT PROLIFERATION

Some chemicals occurring naturally within plant tissues (i.e. endogenously), have a regulatory, rather than a nutritional role in growth and development. These compounds, which are generally active at very low concentrationsare known as plant hormones (or plant growth substances). Synthetic chemicals with similar physiological activities to plant growth substances or compounds having an ability to modify plant growth by some other means for example polyamines are usually termed plant growth regulators. Some of the natural growth substances are prepared synthetically or through fermentation processes and can be purchased from chemical suppliers. When these

chemicals have been added to plant tissue culture media, they are termed plant growth regulators. Plant growth regulator is indispensable material in culture media and is important to the plant tissue induction, organ differentiation and growth. There are several recognized classes of plant growth substance. Until relatively recently only five groups were recognised namely: auxins, cytokinins, gibberellins, ethylene, abscisic acid. Auxins and cytokinins are the most important for regulating growth and morphogenesis in plant tissue cultures. (George *et al.,* 2008).

5.1.1 Effect of cytokinin

Cytokinins comprise a separate class of growth substances and growth regulators. They produce various effects when applied to intact plants. These stimulate protein synthesis and participate in cell cycle control, as they can promote the maturation of chloroplasts and delay the senescence of detached leaves.The effect of cytokinins is most noticeable in tissue cultures where they are used, often together with auxins, to stimulate cell division and control morphogenesis. When added to shoot culture media, these compounds overcome apical dominance and release lateral buds from dormancy (George *et al.,* 2008).

Literature is replete with examples where cytokinins have been found to be effective for the induction of multiple shoots from auxiliary/apical explants, obviously due to the presence of pre-existence of meristems. In fact such a property has been fully exploited for micropropagation in the species.

BAP was used as cytokinin for shoot bud proliferation in the present investigation, the medium devoid of growth regulatorsfailed to initiate bud break or callus from any of the explant. Perusal of Table 4.1

indicates that BAP when incorporated singly in the basal MS medium, induced multiple shoot buds at all the levels, however the most profuseshoot bud breaks (12.8) were observed at 4.0 mg/l BAP.Slight to medium loose and semi compact callus was also induced at the base of auxiliary explants incubated at different levels of BAP (1.5-2.5 mg/l).Lower (0.5-1.0 mg /l) and higher (3.0-6.0) levels of BAP inhibited callus differentiation.

These results were in close agreement with other reports of micropropagation of *Aloe vera* (Hirimburegama and Gamage, 1995, Zhou *et al,* 1999,Chaudhari and Mukundan, 2001, Wu and Xie, 2002, Wang *et al.,* 2002, Aggarwal and Barna, 2004, Baksha *et al.*, 2005,Ahmed *et al.,* 2007, Singh *et al.,* 2009 and Dwivedi *et al.,* 2014). Abrie and Staden (2001) in *Aloe polyphylla* and Eufrocinio and Malasa (2005) in *A. barbadensis* reported best multiple shoot bud induction at medium containing 1.0 mg/l BAP. However, these results were contrary to the results of present investigation with the level of BAP. This might be due to difference in genotypes and explants used in the particular study. Callusing was also observed at the base of auxiliary explant in present investigation which was similar to the observations of Kawai *et al.*, 2006 and Yadav, 2008. They reported callus formation in *Aloe arborescens* and *Aloe vera*.

5.1.2 Interactive effect of cytokinin and auxin

Auxins are very widely used in plant tissue culture and usually form an integral part of nutrient media. An auxin is almost invariably required to promote the initial growth of meristem and shoot tip explants. A low concentration of auxin is often beneficial in conjunction with high levels of cytokinin at Stage II when shoot multiplication is required, although in some cases cytokinin alone is sufficient. It is

important to choose an auxin at a concentration that will promote growth without inducing callus formation.

The word auxin has a Greek origin auxein means to enlarge or to grow. At basic level auxins control cell division and elongation. Since they are capable of initiating cell division they are involved in the formation of meristems giving rise to either unorganised tissue, or defined organs. In organised tissues, auxins are involved in the establishment and maintenance of polarity and in whole plants their most marked effect is the maintenance of apical dominance and mediation of tropisms.

In current investigation addition of auxin to cytokinin containing media enhanced the shoot bud multiplication considerably (Table 4.2). Highest shoot proliferation (12.6) was observed at medium supplimented with 4.5 mg/l BAP + 0.6 mg/l NAA. These were at par with the level of BAP (4.0 mg/l) where maximum shoot proliferation was observed upon supplementation in the medium alone. Callus formation was completely inhibited on the base of explants in the medium supplemented with combination of cytokinin (BAP) and auxin (NAA).

These results are in accordance with the results obtained by Khanam and Sharma(2014). They reported that a perfect combination of auxin and cytokinin is needed for optimum shoot induction. MS basal medium in combination with 4.0 mg/l BAP and 0.2 mg/l NAA was found to be the best on which explants began to show emergence of shoot buds within one week.

The results of present investigation were also similar to the report of Baksha *et al.* (2005), Nayankantha *et al.(*2010) and Kiran*et al. (* 2017), who reported synergetic effect of BAP and NAA in shoot bud break.

Molsaghi *et al.,* (2014) found maximum proliferation on MS medium containing 4.0 mg/l BAP + 1.0 mg/l IAA and 0.8 mg/l BAP + 0.2 mg/l IAA indicates that it is an appropriate combination of medium for producing multiple shoots from single explants. However, the variations among the results of shoot proliferation might be due to environmental and cultural differences. Although callusing results are in contrast to other reports (Sarkar and Roy, 1984, Roy and Sarkar, 1991) of callusing in *Aloe vera* where NAA + BAP gave better results. This might be due to difference in medium and explants.

5.2 ROOT INDUCTION

The induction of rhizogenesis usually requires an adjustment in the levels of auxins and cytokinins. Rhizogenesis is usually achieved by treatment with auxin alone. Also, development of lateral roots is stimulated by auxin as was demonstrated in *Panax ginseng*, where IBA was shown to be more effective than NAA (Kim *et al.,* 2003). Exogenous cytokinins are commonly inhibitory (Reid and Howell, 1995).

To standardize the induction of roots, MS medium supplemented with 0.5-2.0 mg/l IBA/NAA in alone and 0.5-2.0 mg/l NAA + 0.5 and 2.0 mg/l IBA in combination were used in the present study. Root induction was observed with 100 per cent frequency at all the levels of auxins added either singly or in combinations. Maximum rooting was observed in the media supplemented with 1.5 mg/l IBA singly and 0.5 mg/l IBA + 2.0 mg/l NAA in combination. In comparison to NAA, IBA produced significantly higher number of roots at almost all the levels. NAA induced thicker but significantly lower number of roots.

Results of present investigation were similar to the reports of Abrie and Staden (2001) in *Aloe polyphylla* with respect to type of plant

growth regulator (IBA), they observed maximum root on medium supplemented with 0.5 mg/l IBA. However, in the present study maximum root induction was ovserved at 1.5 mg/l IBA. This might be due to difference in the species under study.

Hashemabadi and Kaviani (2008) found the best root induction at MS medium supplemented with 1.0 mg/l IBA + 1.0 mg/l NAA. Khanam and Sharma, (2014) reported profuse root induction after one week of culture at 2.0 mg/l IBA and 1.0 mg/l NAA. However, these levels were not tested in the present investigation.

The enhancing role of IBA on *in vitro* root induction has been reported earlier by Drazeta,1997 in pomegranate, Asghar *et al.* (2011) in orchid, Rafique *et al.* (2012) in dendrobium orchid,Lakshmi *et al.* (2013) in *Hoya wightii,*Shekhawat*et al*. (2015) in *Morinda citrifolia*, Lizarraga *et al.* (2017) in apple and pear cultivars and Meneguzzi *et al.* (2017) in apple.

Our results revealed that IBA could be used as a good root inducer, which was supported by the findings of Amin *et al.* (2003) in *Paederia foetida* and Zhang *et al*. (2004) in *Allium cepa*.

Many workers advocated role of IAA (0.1 mg/l) for rooting under *in vitro* cultures *viz.,* Chaudhuri and Mukundan (2001) in *Aloe vera,* Mouhamad *et al.* (2014) in *Sesbania grandiflora* andGandhi *et al*. (2018) in *Aegle marmelos.* However, in present investigation the effect of IAA was not assessed.

5.3 EFFECT OF EXPLANTS

Plant tissue cultures are initiated from tiny pieces, called explants, taken from any part of a plant. Practically all parts of a plant have been used successfully as a source of explants. In practice, the

"explant" is removed surgically, surface sterilized and placed on a nutrient medium to initiate the mother culture, that is multiplied repeatedly by subculture.The type of explants, size, position, age, physiological state and the manner in which it is cultured can affect the initiation of the cultures and further morphogenetic response (Murashige and Skoog, 1974).

In present investigation apical shoot and auxiliary shoot explants were inoculated at the most responsive levels of plant growth regulators for shoot proliferation and root induction.

Perusal of data from Table 4.5 and Table 4.6 auxiliary shoot explants showed better shoot proliferation (11.0 shoots with 9.65 cm length and 12.8 shoots with 8.58 cm length) at media supplemented with 4.0 mg/l BAP and 4.5 mg/l BAP + 0.6 mg/l NAA, respectively.

Results of present investigation were similar with the observation of Abdi *et al.* (2013) in *Aloe vera* for shoot proliferation in auxiliary shoots along with sheath. They obtained maximum shoot proliferation in the MS medium supplemented with 4.0 mg/l BAP.

Similar pattern of results was found for root induction also where auxiliary shoot explants exhibited significantly better results as compared to apical shoot explants. These results indicated that auxiliary shoot explants were significantly better than apical explants for both shoot proliferation and root induction.

5.4 EFFECT OF GENOTYPES

The rate and response of micropropagation is predominantly dependent on genotype from which explant was taken. It is known that propagation rate is genetically controlled. The results of present investigation revealed that genotypes JA-1, JA-2 and JA-3 differed

significantly for shoot proliferation as well as for root induction. When auxiliary shoot explants inoculated on MS medium supplemented with the most responsive levels of plant growth regulators, induced different response percentages for shoot proliferation (Table 4.7). The better response for shoot proliferation was obtained from JA-1 (100 per cent) as compared to JA-2 (90 per cent) and JA-3 (60 per cent) at 4.0 mg/l BAP.

All the genotypes exhibited 100 per cent rooting response at both 1.5 mg/l IBA and 2.0 mg/l IBA + 0.5 mg/l NAA. JA-2 showed the most profuse rooting (Table 4.8).These results are similar with the earlier observations ofTyagi and Prakash (2004) in jojoba, Sanatombi and Sharma (2008) in capsicum, Payghamzadeh and Kazemitadar (2011) in walnut and Mangena (2018) in soybean for shoot proliferation.

5.5 ACCLIMATIZATION OF PLANTLETS

Acclimatization is the climate adaptation of an organism especially a plant, which has been moved to a new environment (Conover and Poole, 1984). Acclimatization of tissue culture plants is the most crucial step in regeneration. The plants produced are very soft to face ambient environmental conditions (Bhojwani and Razdan, 1992). These plants are grown under controlled conditions. Under these conditions the leaves of plants develop cuticle and its photosynthetic system starts functioning.

In the present study, after 40-50 days of culture of *in vitro* developed shoots on most responsive rooting media wereshifted to pots filled with mixture of 1:1 ratio of soil and vermicompost for their hardening prior to final transfer to soil, showed good percentage of survival (80 per cent) in culture room. In shade house plants also

showed 74 per cent survival rate. The growth and elongation of plants were less in culture room whereas, in shade house growth of plants was better and they also started to elongate. The leaves also started to thicken in shade house.

Perusal of literature on micropropagation of *Aloe* also indicated that rooting and hardening had never been encountered as a problem in *Aloe's* tissue culture. Various authors have reported successfully *in vitro* rooting (Wang *et al.* 2002, Aggarwal and Barna, 2004, Singh and Sood, 2009, Hashemabadi and Kaviani, 2008, Kalimuthu *et al.,* 2010 and Rathore *et al.*, 2011) and similar findings were observed in present study.

In the present investigation we did not investigate all the factors which in one way or other way block or stimulate micropropagation potential in *Aloe vera*.thus in the light of these factors we feel the multiprolonged and intensive *in vitro* efforts are needed to exploit the micropropagation in *Aloe vera.*

Chapter- 5

DISCUSSION

Tissue culture is the cultivation of plant cells, tissues and organs on specially formulated nutrient media.Over 100 years ago, Haberlandt envisioned the concept of plant tissue culture and provided the groundwork for the cultivation of plant cells, tissues and organs in culture. It relies on phenomenon of cell totipotency, the latter being the ability of single cells to divide, produce all the differentiated cell characteristics of organs and regenerate into a whole plant. The technique of plant tissue culture is used for growing isolated plant cells, tissues and organs under axenic conditions (*in vitro*) to regenerate and propagate entire plants.

Tissue culture techniques include embryo culture, anther culture, induction of somaclonal variations, micropropagation, protoplast culture, cryopreservation, production of synthetic seed and production of secondary metabolites. Among these cultures micropropagation has become an important part of the commercial propagation of many plants because of its advantages as a multiplication system. Fertile plants can be regenerated either by the growth and proliferation of existing auxiliary and apical meristems or by the regeneration of adventitious shoots. Adventitious buds and shoots are formed *de novo,*meristems are initiated from explants, such as those of leaves petioles, hypocotyls, floral organs and roots. (Iliev *et al.,* 2010)

Today plant tissue culture applications encompass much more than clonal propagation. The range ofroutine technologies has expanded to include somatic embryogenesis, somatic hybridization,

viruselimination as well as the application of bioreactors to mass propagation.Plant tissue culture technique though an underutilized tool, it can be extensively applied in horticulture and agriculture to increase crop production.

Plant tissue culture is now a well-established technology.Like many other technologies, it has gone through different stages of evolution, scientific curiosity, researchtool, novel applications and mass exploitation. Initially,plant tissue culture was exploited as a research tool andfocused on attempts to culture and study the developmentof small, isolated segments of plant tissues orisolated cells. Around the mid twentieth century, thenotion that plants could be regenerated or multiplied fromeither callus or organ culture was widely accepted andpractical application in the plant propagation industryensued. The technique was heralded as the universalmass clonal plant propagation system for the future andthe term 'micropropagation' was introduced to describe more accurately the processes (Akin-Idowu *et al.,* 2009).

However, the capacity to propagate '*in vitro*' is generally influenced by genotypes of particular species, tissues of specific organs, appropriate chemical and physical environment used for culturing. Most of the researchers try to use optimum physical and physiological conditions for proper growth of cells, tissues, organs and plants, empirically manipulate ratio of plant growth regulators to affect cell proliferation and differentiation.

Demand of *Aloe vera,* has increased many fold in recent past due to its highly priced cosmetic and medicinal properties and it is being used almost in every cosmetic product these days (Cera *et al.,* 1980, Davis and Leiter,1988, Afzal *et al.,* 1991 and Reynolds and Dweck,1999). In nature, it propagates vegetatively but the rate is very

slow and is highly dependent on climatic conditions. This species is also affected by a widespread male sterility (Keijzer and Cresti, 1987). Thus the availability of quality and true to type planting material is going to be a limiting factor in *Aloe* cultivation in near future. Perusal of review reveals that micropropagation of members of *Aloe* have been influenced by the type of plant growth regulators and their levels used either singly or in combinations.

In the present investigation, attempts were made to optimize the conditions for shoot multiplication through the manipulation of plant growth regulators and assess the effect of different explants and genotypesunder *in vitro* conditions of *Aloe vera.*

While discussing the results, efforts have been made to compare and analyse these with the work already published. Since voluminous literature is available on totipotency of plant cells, it is not possible to refer all the previous interpretation. However, relevant references have been cited both from related and other genera.

5.1 EFFECT OF PLANT GROWTH REGULATORS ON SHOOT PROLIFERATION

Some chemicals occurring naturally within plant tissues (i.e. endogenously), have a regulatory, rather than a nutritional role in growth and development. These compounds, which are generally active at very low concentrationsare known as plant hormones (or plant growth substances). Synthetic chemicals with similar physiological activities to plant growth substances or compounds having an ability to modify plant growth by some other means for example polyamines are usually termed plant growth regulators. Some of the natural growth substances are prepared synthetically or through fermentation processes and can be purchased from chemical suppliers. When these

chemicals have been added to plant tissue culture media, they are termed plant growth regulators. Plant growth regulator is indispensable material in culture media and is important to the plant tissue induction, organ differentiation and growth. There are several recognized classes of plant growth substance. Until relatively recently only five groups were recognised namely: auxins, cytokinins, gibberellins, ethylene, abscisic acid. Auxins and cytokinins are the most important for regulating growth and morphogenesis in plant tissue cultures. (George *et al.,* 2008).

5.1.1 Effect of cytokinin

Cytokinins comprise a separate class of growth substances and growth regulators. They produce various effects when applied to intact plants. These stimulate protein synthesis and participate in cell cycle control, as they can promote the maturation of chloroplasts and delay the senescence of detached leaves.The effect of cytokinins is most noticeable in tissue cultures where they are used, often together with auxins, to stimulate cell division and control morphogenesis. When added to shoot culture media, these compounds overcome apical dominance and release lateral buds from dormancy (George *et al.,* 2008).

Literature is replete with examples where cytokinins have been found to be effective for the induction of multiple shoots from auxiliary/apical explants, obviously due to the presence of pre-existence of meristems. In fact such a property has been fully exploited for micropropagation in the species.

BAP was used as cytokinin for shoot bud proliferation in the present investigation, the medium devoid of growth regulatorsfailed to initiate bud break or callus from any of the explant. Perusal of Table 4.1

indicates that BAP when incorporated singly in the basal MS medium, induced multiple shoot buds at all the levels, however the most profuseshoot bud breaks (12.8) were observed at 4.0 mg/l BAP.Slight to medium loose and semi compact callus was also induced at the base of auxiliary explants incubated at different levels of BAP (1.5-2.5 mg/l).Lower (0.5-1.0 mg /l) and higher (3.0-6.0) levels of BAP inhibited callus differentiation.

These results were in close agreement with other reports of micropropagation of *Aloe vera* (Hirimburegama and Gamage, 1995, Zhou *et al,* 1999,Chaudhari and Mukundan, 2001, Wu and Xie, 2002, Wang *et al.,* 2002, Aggarwal and Barna, 2004, Baksha *et al*., 2005,Ahmed *et al.,* 2007, Singh *et al.,* 2009 and Dwivedi *et al.,* 2014). Abrie and Staden (2001) in *Aloe polyphylla* and Eufrocinio and Malasa (2005) in *A. barbadensis* reported best multiple shoot bud induction at medium containing 1.0 mg/l BAP. However, these results were contrary to the results of present investigation with the level of BAP. This might be due to difference in genotypes and explants used in the particular study. Callusing was also observed at the base of auxiliary explant in present investigation which was similar to the observations of Kawai *et al*., 2006 and Yadav, 2008. They reported callus formation in *Aloe arborescens* and *Aloe vera*.

5.1.2 Interactive effect of cytokinin and auxin

Auxins are very widely used in plant tissue culture and usually form an integral part of nutrient media. An auxin is almost invariably required to promote the initial growth of meristem and shoot tip explants. A low concentration of auxin is often beneficial in conjunction with high levels of cytokinin at Stage II when shoot multiplication is required, although in some cases cytokinin alone is sufficient. It is

important to choose an auxin at a concentration that will promote growth without inducing callus formation.

The word auxin has a Greek origin auxein means to enlarge or to grow. At basic level auxins control cell division and elongation. Since they are capable of initiating cell division they are involved in the formation of meristems giving rise to either unorganised tissue, or defined organs. In organised tissues, auxins are involved in the establishment and maintenance of polarity and in whole plants their most marked effect is the maintenance of apical dominance and mediation of tropisms.

In current investigation addition of auxin to cytokinin containing media enhanced the shoot bud multiplication considerably (Table 4.2). Highest shoot proliferation (12.6) was observed at medium supplimented with 4.5 mg/l BAP + 0.6 mg/l NAA. These were at par with the level of BAP (4.0 mg/l) where maximum shoot proliferation was observed upon supplementation in the medium alone. Callus formation was completely inhibited on the base of explants in the medium supplemented with combination of cytokinin (BAP) and auxin (NAA).

These results are in accordance with the results obtained by Khanam and Sharma(2014). They reported that a perfect combination of auxin and cytokinin is needed for optimum shoot induction. MS basal medium in combination with 4.0 mg/l BAP and 0.2 mg/l NAA was found to be the best on which explants began to show emergence of shoot buds within one week.

The results of present investigation were also similar to the report of Baksha *et al.* (2005), Nayankantha *et al.(*2010) and Kiran*et al. (* 2017), who reported synergetic effect of BAP and NAA in shoot bud break.

Molsaghi *et al.,* (2014) found maximum proliferation on MS medium containing 4.0 mg/l BAP + 1.0 mg/l IAA and 0.8 mg/l BAP + 0.2 mg/l IAA indicates that it is an appropriate combination of medium for producing multiple shoots from single explants. However, the variations among the results of shoot proliferation might be due to environmental and cultural differences. Although callusing results are in contrast to other reports (Sarkar and Roy, 1984, Roy and Sarkar, 1991) of callusing in *Aloe vera* where NAA + BAP gave better results. This might be due to difference in medium and explants.

5.2 ROOT INDUCTION

The induction of rhizogenesis usually requires an adjustment in the levels of auxins and cytokinins. Rhizogenesis is usually achieved by treatment with auxin alone. Also, development of lateral roots is stimulated by auxin as was demonstrated in *Panax ginseng*, where IBA was shown to be more effective than NAA (Kim *et al.,* 2003). Exogenous cytokinins are commonly inhibitory (Reid and Howell, 1995).

To standardize the induction of roots, MS medium supplemented with 0.5-2.0 mg/l IBA/NAA in alone and 0.5-2.0 mg/l NAA + 0.5 and 2.0 mg/l IBA in combination were used in the present study. Root induction was observed with 100 per cent frequency at all the levels of auxins added either singly or in combinations. Maximum rooting was observed in the media supplemented with 1.5 mg/l IBA singly and 0.5 mg/l IBA + 2.0 mg/l NAA in combination. In comparison to NAA, IBA produced significantly higher number of roots at almost all the levels. NAA induced thicker but significantly lower number of roots.

Results of present investigation were similar to the reports of Abrie and Staden (2001) in *Aloe polyphylla* with respect to type of plant

growth regulator (IBA), they observed maximum root on medium supplemented with 0.5 mg/l IBA. However, in the present study maximum root induction was ovserved at 1.5 mg/l IBA. This might be due to difference in the species under study.

Hashemabadi and Kaviani (2008) found the best root induction at MS medium supplemented with 1.0 mg/l IBA + 1.0 mg/l NAA. Khanam and Sharma, (2014) reported profuse root induction after one week of culture at 2.0 mg/l IBA and 1.0 mg/l NAA. However, these levels were not tested in the present investigation.

The enhancing role of IBA on *in vitro* root induction has been reported earlier by Drazeta,1997 in pomegranate, Asghar *et al.* (2011) in orchid, Rafique *et al.* (2012) in dendrobium orchid,Lakshmi *et al.* (2013) in *Hoya wightii,*Shekhawat*et al*. (2015) in *Morinda citrifolia*, Lizarraga *et al.* (2017) in apple and pear cultivars and Meneguzzi *et al.* (2017) in apple.

Our results revealed that IBA could be used as a good root inducer, which was supported by the findings of Amin *et al.* (2003) in *Paederia foetida* and Zhang *et al*. (2004) in *Allium cepa*.

Many workers advocated role of IAA (0.1 mg/l) for rooting under *in vitro* cultures *viz.,* Chaudhuri and Mukundan (2001) in *Aloe vera,* Mouhamad *et al.* (2014) in *Sesbania grandiflora* andGandhi *et al*. (2018) in *Aegle marmelos.* However, in present investigation the effect of IAA was not assessed.

5.3 EFFECT OF EXPLANTS

Plant tissue cultures are initiated from tiny pieces, called explants, taken from any part of a plant. Practically all parts of a plant have been used successfully as a source of explants. In practice, the

"explant" is removed surgically, surface sterilized and placed on a nutrient medium to initiate the mother culture, that is multiplied repeatedly by subculture.The type of explants, size, position, age, physiological state and the manner in which it is cultured can affect the initiation of the cultures and further morphogenetic response (Murashige and Skoog, 1974).

In present investigation apical shoot and auxiliary shoot explants were inoculated at the most responsive levels of plant growth regulators for shoot proliferation and root induction.

Perusal of data from Table 4.5 and Table 4.6 auxiliary shoot explants showed better shoot proliferation (11.0 shoots with 9.65 cm length and 12.8 shoots with 8.58 cm length) at media supplemented with 4.0 mg/l BAP and 4.5 mg/l BAP + 0.6 mg/l NAA, respectively.

Results of present investigation were similar with the observation of Abdi *et al.* (2013) in *Aloe vera* for shoot proliferation in auxiliary shoots along with sheath. They obtained maximum shoot proliferation in the MS medium supplemented with 4.0 mg/l BAP.

Similar pattern of results was found for root induction also where auxiliary shoot explants exhibited significantly better results as compared to apical shoot explants. These results indicated that auxiliary shoot explants were significantly better than apical explants for both shoot proliferation and root induction.

5.4 EFFECT OF GENOTYPES

The rate and response of micropropagation is predominantly dependent on genotype from which explant was taken. It is known that propagation rate is genetically controlled. The results of present investigation revealed that genotypes JA-1, JA-2 and JA-3 differed

significantly for shoot proliferation as well as for root induction. When auxiliary shoot explants inoculated on MS medium supplemented with the most responsive levels of plant growth regulators, induced different response percentages for shoot proliferation (Table 4.7). The better response for shoot proliferation was obtained from JA-1 (100 per cent) as compared to JA-2 (90 per cent) and JA-3 (60 per cent) at 4.0 mg/l BAP.

All the genotypes exhibited 100 per cent rooting response at both 1.5 mg/l IBA and 2.0 mg/l IBA + 0.5 mg/l NAA. JA-2 showed the most profuse rooting (Table 4.8).These results are similar with the earlier observations ofTyagi and Prakash (2004) in jojoba, Sanatombi and Sharma (2008) in capsicum, Payghamzadeh and Kazemitadar (2011) in walnut and Mangena (2018) in soybean for shoot proliferation.

5.5 ACCLIMATIZATION OF PLANTLETS

Acclimatization is the climate adaptation of an organism especially a plant, which has been moved to a new environment (Conover and Poole, 1984). Acclimatization of tissue culture plants is the most crucial step in regeneration. The plants produced are very soft to face ambient environmental conditions (Bhojwani and Razdan, 1992). These plants are grown under controlled conditions. Under these conditions the leaves of plants develop cuticle and its photosynthetic system starts functioning.

In the present study, after 40-50 days of culture of *in vitro* developed shoots on most responsive rooting media wereshifted to pots filled with mixture of 1:1 ratio of soil and vermicompost for their hardening prior to final transfer to soil, showed good percentage of survival (80 per cent) in culture room. In shade house plants also

showed 74 per cent survival rate. The growth and elongation of plants were less in culture room whereas, in shade house growth of plants was better and they also started to elongate. The leaves also started to thicken in shade house.

Perusal of literature on micropropagation of *Aloe* also indicated that rooting and hardening had never been encountered as a problem in *Aloe's* tissue culture. Various authors have reported successfully *in vitro* rooting (Wang *et al.* 2002, Aggarwal and Barna, 2004, Singh and Sood, 2009, Hashemabadi and Kaviani, 2008, Kalimuthu *et al.,* 2010 and Rathore *et al.*, 2011) and similar findings were observed in present study.

In the present investigation we did not investigate all the factors which in one way or other way block or stimulate micropropagation potential in *Aloe vera*.thus in the light of these factors we feel the multiprolonged and intensive *in vitro* efforts are needed to exploit the micropropagation in *Aloe vera.*

Chapter- 6

SUMMARY AND CONCLUSION

Aloe vera(L.)Burm.is a xerophytic medicinal plant of considerable importance. It is widely used in cosmetic and drug industry and its demand is increasing day by day. It requires well-drained soil and can grow in nutritionally poor soil.Due to widespread male sterility it propagates only through vegetative mode of reproduction. Propagation of *Aloe vera* is primarily by means of offshoots, but propagation rate is very slow as a single plant can produce 3-4 offshoots in a year and is not able to produce required number of plants for undertaking commercial plantations.

Hencethe production of *Aloe* leaves is insufficient to meet the industry demand. Due to slow rate of natural growth, ever increasing demand for this “Potted Physician” cannot meet with traditional method of propagation, hence there is need for mass propagation of this plant through *in vitro* method to fulfil the demand of pharmaceutical and cosmetic industries.So keeping this thing in mind, micropropagation work was carried out on this plant with the following objectives-

(iv) To determine the optimum level of different plant growth regulators for rapid shoot multiplication of *Aloe vera.*

(v) To study the effects of genotypes and explants on micropropagation of *Aloe vera.*

(vi) To standardize protocol for rooting and hardening of micropropagated plantlets.

Different explants *viz*.,auxiliary shootsand apical shoot explants were obtained from nursery of *Aloe vera*accessions beingmaintained at Department of Plant Breeding and Genetics, S.K.N. College of Agriculture, Jobner.Murashige and Skoog (1962) medium was used throughout the course of investigation. Explants were subjected to varying concentrations of various plant growth regulators such as cytokinin (BAP) and auxins (IBA and NAA). The cultures were

incubated in culture room maintained at 25±2°C temperature and 14:10 hours photoperiod with a light intensity of 3000 lux.

Following conclusions were drawn from this study:-

1. MS medium supplemented singly with 4.0 mg/l BAPwas found best for shoot bud break.
2. MS medium supplemented with 4.5 mg/l BAP and 0.6 mg/l NAA in combination were found best for shoot bud break.
3. MS medium supplemented singly with 1.5 mg/l IBA was found best for root induction.
4. MS medium supplemented with 2.0 mg/l IBA and 0.5 mg/l NAA in combination were found best for shoot bud break.
5. Auxiliary shoot explant was found best for shoot proliferation and root induction.
6. *Aloe vera* genotype JA-1 was found the most responsive for shoot proliferation and genotype JA-2 was most responsive for root induction.

On the basis of present investigation, it is recommended that the above given protocols may be used for mass micropropagation of *Aloevera.*

LITERATURE CITED

Abdi, G., Hedayat, M. and Modarresi, M. 2013.*In vitro* micropropagation of *Aloe vera*– Impacts of plant growth regulators, media and type of explants. *Journal of Biological and Environmental Sciences,***7**(9): 19-24.

Abrie, A. and Staden, J. V. 2001. Micropropagation of endangered *Aloe polyphylla.Plant Growth Regulation*, **33**(1): 19-23.

Adams, S. P., Leitch, I. J., Bennett, M. D., Chase, M. W. and Leitch, A. R. 2000.Ribosomal DNA evolution and phylogeny in *Aloe* (Asphodelaceae).*American Journal of Botany,* **87(11):** 1578–1583.

Addicott, F. T. 1982.Abscission. *Berkeley: University of California Press*.

Afzal, M., Ali, M., Hassan, R. A. H., Sweedan, N. and Dhami, M. S. I. 1991. Identification of some prostnoids in *Aloe vera* extracts. *Planta Medica,***57**: 38-40.

Aggarwal, D. and Barna, K. S. 2004.Tissue culture propagation of elite plant of *Aloevera*(L.)*Journal of Plant Biochemistry and Biotechnology,***13**: 77-79.

Ahmed, S., Kabir, A. H., Ahmed, M. B., Razy, M. A. and Ganesan, S. 2007. Development of rapid micropropagation method of *Aloe vera* (L).*Sjemenarstvo***, 24** : 2.

Akin-Idowu, P. E., Ibitoye, D. O. and Ademoyegun, O. T. 2009.Tissue culture as a plant production technique for horticultural crops.*African Journal of Biotechnology,***8**(16): 3782-3788.

Alagumanian, S., Saravanaperumal, V., Balachandar, R., Rameshkannan, K. and Rao, M. V. 2004. Plant regeneration from leaf and stem explants of *Solanum trilobatum* L. *Current Science*, **86**: 1478-1480.

Alezadeh, M., Singh, S. K. and Patel, V. B. 2009.Comparative performance of *in vitro* multiplication in four grape (*Vitis* spp.) rootstock genotypes.*International Journal of Plant Production,* **4** (1):1735-8043.

Ali, S. and Mirza, B. 2006.Micropropagation of rough lemon *Citrus jambhiri* Lush.Effect of explant type and hormone concentration.*Acta Botanica Croatica*, **65**: 137-146.

Aloni, R. 1995. The induction of vascular tissue by auxin and cytokinin.*Plant Hormones: Physiology, Biology and Molecular Biology,* **1***:* 531-546.

Amin, M. N., Rahman, M. M. and Manik, M. S. 2003. *In vitro* clonal propagation of *Paederia foetida* L.: A medicinal plant of Bangladesh. *Plant Tissue Culture,***13:** 117–123.

Asghar, S., Ahmad, T., Ahmad, I. and Yaseen, M. 2011.*In vitro* propagation of orchid (*Dendrobium nobile*) var. Emma white. *African Journal of Biotechnol*ogy, **10:** 3097-3103.

Baksha, R., Jahan, M. A. A., Khatun, R. and Munshi, J. L. 2005.Micropropagation of *Aloe barbadensis* Mill.through*in vitro* culture of shoot tip explants. *Plant Tissue Culture and Biotechnology,* **15**(2): 121-126.

Banerjee, P., Maity, S. A., Maiti, S. S. and Banerjee, N. 2007. Influence of genotype on *in vitro* multiplication potential of *Arachis hypogaea* L. *Acta Botanica Croat*ica, **66**(1) : 15-23.

Bensaad, Z. M. and Milad, K. M. 2015. Influence of type of explant, salt composition of basal medium and cytokinins on micropropagation of pomegranate (*Punica granatum* Khadouri). *Acta Horticulturae,* 1089: 91-96.

Bhandari, A. K., Negi, J. S., Bisht, V. K. and Bharti, M. K., 2010. *In vitro* propagation of *Aloe vera*: A plant with medicinal properties. *Nature and Science,* **8**(8): 174-176.

Bhojwani, S. S. and Razdan, M. K. 1992. Plant tissue culture: Theory and practice. *Biological Science,***39(1)**: 1- 9.

Bhojwani, S. S. and Razdan, M. K. 1996. In Plant Tissue Culture: Theory and Practice. Elsevier , Amersterdam, London, New York, Tokyo.

Biswas, G. C., Miah, M., Sohel, H. M., Hossain, A. K. M., Shakil, S. K. and Howlader, M. S. 2013. Micro propagation of *Aloe indica* L. through shoot tip culture.*Journal of Agriculture and Veterinary Science,***5**: 30-35.

Bonyanpour, A. and Khui, M. K. 2013. Callus induction and plant regeneration in *Punica granatum* L. ‘Nana’ from leaf explants. *Journal of Central European Agriculture,* **14**(3): 75-83.

Campestrini, L. H., Kuhnen, S., Lemos, P. M. M., Bach, D. B., Dias, P. F. and Maraschin, M. 2006. Cloning protocol of *Aloe vera* as a study-case for “Tailor-Made” biotechnology to small farmers.*Journal of Technology Management and Innovation*, **1(5):** 76-79.

Cera, L. M., Heggers, J., Robson, M. C. and Hafstrom, W. J. 1980. The therapeutic efficacy of *Aloe vera* cream in thermal injuries, two case reports. *Journal of the American Animal Hospital Association*,**16**: 768-772.

Chandler, S. F. and Thorpe, T. A. 1986.Hormonal regulation of organogenesis *invitro*.*Hormonal regulation of plant growth and development*, India: Agro Botanical Publication, **3:** 1-27.

Chandra, R., Khetarpal, S., Patil, P., Gupta, N. and Polistly, R. 1995.*In vitro* regeneration of hybrid and non hybrid tomato (*Lycopersicon esculantum* L.).*Indian Journal of Plant Pathology*, **37**(2): 139-142.

Chaudhuri, S. and Mukundan, U. 2001.*Aloe vera*L. micropropagation and characterization of its gel. *Phytomorphology*, **51**: 155-157.

Chaugule, R. R., More, T. A., Patil, R. S. and Kamble, A. B. 2005. Callus culture for rapid regeneration of pomegranate. *Journal of Maharashtra Agricultural Universities,* **30**(1): 85-86.

Choudhary, A. K., Ray, A. K. , Jha, S. and Mishra, I. N. 2011. Callus formation, shoot initiation and *in vitro* culture of *Aloe vera*. *Biotechnology, Bioinformatics and Bioengineering,* **1**(4): 551-553.

Chukwujekwu, J. C., Fennell, C. W. and Staden, J. V. 2002. Optimisation of the tissue culture protocol for the endangered *Aloe polyphylla. South African Journal of Botany,* **68:** 424–429.

Conover, C. A. and Poole, R. T., 1984.Growth of *Calathea makoyana* as influence by media, fertilizer and irrigation.*University of Florida Institute of Food and Agriculture Sciences*

Corneanu, M., Corneanu, G., Vekas, M. and Minea, R. 1994.*In vitro* organogenesis of *Aloe arborescencs*.*Revue Roumaine de Biologie,***39** (1) : 45-52.

Das, A., Mukherjee, P. and Jha, T. B. 2010. High frequency micropropagation of *Aloe vera* L. Burm. f. as a low cost option towards commercialization. *Plant Tissue Culture and Biotechnology,* **20**(1): 29-35.

Davies, P. J. 1995. Plant hormones. *Dordrecht: Kluwer Academic Publishers*: 13-38.

Davis, R. H. and Leiter, M. G. 1988.*Aloe vera*: A natural approach for treating wounds, edema and pain diabetes. *Journal of the American Pediatric Medical Association*.**78**(2): 60-68.

Debergh, P. C. and Read, P. E. 1991.Micropropagation.*Micropropagation Technology and Application,* pp: 1-13.

Drazeta, L. 1997. Pomegranate (*Punica granatum* L.) propagation by *in vitro* method of tissue culture.Review of Research Work at the Faculty of Agriculture, Belgrade **42**(1): 49-59.

Dwivedi, N. K., Indiradevi, A., Asha, K. I., Nair, R. A. and Suma, A. 2014. A protocol for micropropagation of *Aloe vera* L. (Indian Aloe)– A miracle plant, *Research in Biotechnology*,**5**(1): 1–5.

Eufrocinio, C. M. and Malasa, A. B. 2005.Tissue culture for the rapid clonal propagation of *Aloe barbadensis* Miller.*The Philippines Agricultural Scientist,* **88**(1): 167-170.

Fabijan, D. M., Plumb-Dhindsa, P. and Reid, D. M. 1981.Effects of two growth retardants on tissue permeability in *Pisum sativum* and *Beta vulgaris*.*Planta,* **152**: 481-486.

Fougat, R. S., Pandya, S. B., Ahmad, T. and Godhani, P. R. 1997.*In vitro* studies in pomegranate (*Punica granatum* L.).*Journal of Applied Horticulture*,**3**(1/2): 23-29.

Gandhi, K., Rajesh, E., Saravanan, S. and Elamvaluthi, M. 2018.Micropropagation of *Aegle marmelos* L. — A medicinal tree through culture of axillary bud and shoot tip explants from *In vitro* germinated seedlings. *International Journal of Scientific Research in Biological Sciences*, **5** (3): 65-71.

Gautheret, R. J. 1985. History of plant tissue and cell culture.A personal account in (Vasi) I.K. ed. Cell culture and somatic cell.*Genetics of Plants, Cell Growth Nutrition Cytodifferentiation and Cryo Preservation Academic Press Inc. Orlando*, **2**: 2-59.

George, E. F. and Sherrington, P. D., 1984.Plant propagation by tissue culture.*Handbook and Directory of Commercial Laboratories, London Exegetics Limited:* 102-110.

George, E. F., Hall, M. A. and Klerk, G. J. D. 2008. Micropropagation: Uses and Methods. *Plant Propagation by Tissue Culture*, **1:** 29–64.

George, E. F., Machakova, I. and Zazimalova, E 2008. Plant growth regulators I: Introduction, auxins, their analogues and inhibitors. *Plant propagation by Tissue Culture,* **3**: 175-204.

George, E. F., Staden, J. V. and Zazimalova, E. 2008. Plant growth regulators II: cytokinins, their analogues and antagonists. *Plant Propagation by Tissue Culture,* **3**: 205-226.

Gheisari, M. and Miri, S. M.2017.*In vitro* callusinduction and bulblet regeneration of Hyacinth(*Hyacinthus orientalis* L.).*Plant Cell Biotechnology and Molecular Biology,***18**(3 and 4): 145-155.

Gomez, K. A., Gomez, A. A. 1984. *Statistical Procedures for Agricultural Research*. IRRI, Phillipines, **2**nd edition.

Gubis, L., Lajchova, Z., Farago, J. and Jurekova, Z. 2003. Effect of genotype and explant type on shoot regeneration in Tomato (*Lycopersicon esculentum* Mill.) *in vitro.Czech Journal of Genetics and Plant Breeding*, **39**: 9-14.

Gupta, S., Sahu, P.K., Sen, D. L. and Pandey, P. *2014.In vitro* Propagation of *Aloe vera* (L.)Burm. *British Biotechnology Journal,* **4(7)**: 806-816

Haque, S. M. and Ghosh, B. (2013). High frequency microcloning of Aloe vera and their true-to-type conformity by molecular cytogenetic assessment of two years old field growing regenerated plants. *Botanical Studies*, **54**(1): 1.

Hashem, A. D. and Kaviani, B. 2010. *In vitro* proliferation of an important medicinal plant *Aloe* – A method for rapid production.*Australian Journal of Crop Science*, **4** (4): 216-222.

Hashemabadi, D. and Kaviani, B. 2008.Rapid micro-propragation of *Aloe vera* L. via shoot multiplication.*African Journal Biotechnology*, **7**(12): 1899-1902.

Hirimburegama, K and Gamage, N. 1995.*In vitro* multiplication of *Aloe vera* meristem tips for mass propagation. *Horticulture Sci*ence,**27**: 15-18.

Hongxing Wang, Faqi Li, Taixia Wang, Jianjun Li, Junying Li, Xiangfu Yang and Jingyuan Li. 2004. *Chong Yao Cai*, **27**(9): 627-628.

Hosseini, R. and Parsa, M. 2007. Micropropagation of *Aloe vera* L. grown in South Iran.*Pakistan Journal of Biological Sciences,* **10**(7):1134-1137.

Huxter, T. J., Reid, D. M. and Thorpe, T. A. 1981. Shoot initiation in light and dark grown tobacco callus: The role of ethylene. *Physiolgia Plantarum,***53:** 319-326.

Iliev, I., Kitin, P. and Funada, R. 2010.Morphological and anatomical study on *in vitro* root formation of silver birch (*Betula pendula* Roth.).*Propagation of Ornamental Plants,***1**: 10-19.

Jakhar, M.L., Gurjar, Y.R., Choudhary, M.R. and Kakralya, B.L. 2012. Regeneration in callus cultures of Ghrithkumari (*Aloe barbadensis* Mill). *Journal of Plant Science Res*earch, **28**(1): 131-136.

Jayakrishna, C., Karthik, C., Barathi, S., Kamalanathan, D. and Indra, A. S. P. 2011. *In vitro* propagation of *Aloe barbadensis Miller*: a miracle herb. *Research in Plant Biology,* **1(5)**: 22-26.

Kalalbandi, B. M. Waskar, D. P., Khandare, V. S. and Gorad, D. S. 2014. Micropropagation studies on pomegranate var. Bhagwa. *Indian Journal of Horticulture,***71**(4): 564-566.

Kalimuthu, K., Vijayakumar, S., Senthilkumar, R.R. and Suresh K. M. 2010. Micropropagation of *Aloe vera* Linn. - A medicinal plant. *International Journal of Biotechnology and Biochemistry*, **6**(3): 405-410.

Kanta, K. and Maheshwari, P. 1963. Test tube fertilization in some angiosperms.*Phytomorphology*, **13**: 230-237.

Kawai, K., Beppu, H., Koike, T., Fujita, K. and Marunouchi, T. 2006.Tissue culture of *Aloe arborescens* Miller var. *natalensis* Berger.*Phytotherapy Research*, **7**(7): S5-S10.

Keijzer, C. J. and Cresti, M. 1987. A comparison of anther tissue development in male sterile *Aloe vera* and male fertile *Aloe ciliaris. Annuals of Botany*, **59** : 533- 542.

Khan, M. R. I., Heyes, J. K. and Cohen, D. 1988. Plant regeneration from oca (*Oxalis tuberosa* M.): The effect of explant type and culture media. *Plant Cell Tissue and Organ Culture*, **14**: 41-50.

Khanam, M. and Sharma, G. K. 2014.Rapid *in vitro* propagation of *Aloe vera* L. with some growth regulators using lateral shoots as explants.*World Journal of Pharmacy and Pharmaceutical Sciences,* **3**(3): 2278-4357.

Kim, S. J., Hahn, E. J., Paek, K. Y. and Murthy, H. N. 2003. Application of bioreactor culture for large scale production of Chrysanthemum transplants. *Acta Horticultura*, 625:187–191.

Kiran, S., Tirkey, A., Jha, Z. and Porte, S. S. 2017. *In vitro* regeneration of *Aloe vera* (*Aloe barbadensis* Mill).*International Journal of Current Microbiology and Applied Sciences,* 6**(11):** 1829-1834.

Krikorian, A. D. 1995. Hormones in tissue culture and micropropagation.*Plant Hormones: Kluwer Academic Publishers*: 774-796.

Kuhnle, J. A., Fuller, G. and Corse, J. 1977. Antisenescent activity ofnatural cytokinins*. Plant Physiology*, **41**: 14-21.

Kumar, N., Vijayanand, K. G. and Reddy, M. P. 2011. Plant regeneration in non-toxic *Jatropha curcas*: Impacts of plant growth regulators, source and type of explants. *Journal of Plant Biochemistry Biotechnology*, **20**: 125-133.

Kumari, A. and Naseem, M. D. 2015. An efficient protocol for micropropagation of a medicinal plant *Aloe vera* L. through organ culture.*Indian Botanical Society,***94**: 118–125.

Kumari, U., Rishi, K., Vishwakarma, Gupta, N., Ruby, Shirgurkar, M. V. and Khan, V. M. 2015. Efficient shoots regeneration and genetic transformation of *Bacopa monnieri*. *Physiology and Molecular Biology of Plants,* **21**(2): 261-267.

Lakshmi, R., Herald, J., Benjamin, F., Kumar, T. S., Garimella, V., Suryanarayana, M. and Rao, M. 2013. Organogenesis from *in*

vitro derived leaf and internode explants of *Hoya wightii* spp palniensis - A vulnerable species of Western Ghats. *Brazilian Archives of Biology and Technology*, **56**(3): 421-430.

Liao, Z., Chen, M., Tan, F., Sun, X. and Tang, K. 2004. Microprogagation of endangered Chinese aloe.*Plant Cell*, *Tissue and Organ Culture,* **76**: 83–86.

Liu, J. H. and Reid, D. M. 1992. Adventitious rooting in hypocotyls of sunflower (*Helianthus annus*) seedlings.The role of changes in endogenous free and conjugated indole-3-acetic acid.*Physiolgia Plantarum,***86**: 285-292.

Lizarraga, A., Fraga, M., Ascasibar, J. and Gonzalez, M. L. 2017. *In vitro* propagation and recovery of eight apple and two pear cultivars held in a germplasm bank. *American Journal of Plant Sciences*, **8:** 2238-2254.

Mangena, P. 2018. The role of plant genotype, culture medium and *Agrobacterium* on soybean plantlets regeneration during genetic transformation*. Transgenic Crops,* 133-156.

Mathew, S. and Abraham, T. E. 2006.*In vitro* antioxidant activity and scavenging effects of *Cinnamomum verum* leaf extract assayed by different methodologies. *Food Chemistry and Toxicology*,**44**: 198-206.

Mehta, S. 2013. *In vitro* multiplication of Aloe vera L. – An important medicinal plant, *Vegetos*, **26**(special): 155–159.

Meneguzzi, A., Gonclaves, M. J., Camargo, S. S., Grimaldi, F., Weber, G. C. and Rufato, L. 2017.Micropropagation of the new apple rootstock 'G. 814'.*Ciencia Rural,* **47.**

Meyer, H. T. and Staden, J. V. 1991.Rapid *in vitro* propagation of *Aloe barbadensis* Mill.*Plant Cell Tissue Organ Culture,***26**: 171-176.

Mittal, P., Devi, R.And Gosal, S. S. 2015.Effect of genotypes and activated charcoal on high frequency *in vitro* plant regeneration in sugarcane.*Indian Jounal of Biotechnology*, **15**: 261-265.

Molsaghi, M., A. Moieni and D. Kahrizi, 2014. Efficient protocol for rapid *Aloe vera* micropropagation.*Pharmaceutical Biology*, **52**: 735-739.

Monge, G.G., Gatica, A.M. and Valdez, M. 2008. Somatic embryogenesis, plant regeneration and acemannan detection in *Aloe (Aloe barbadensis* Mill).*Agronomia costarricense*, **32**:41–52.

Mouhamad, R. S., Yousir, S. A., Fadhel, A. S., Taha, D. I. and Iqbal, M. 2014.*In vitro* culture and plant regeneration of *Sesbania grandiflora. International Journal of Chemical and Biochemical Sciences,* **6**: 45-49.

Murashige, T. and Skoog, F. 1974. Plant propagation through tissue cultures, *Annual. Review of Plant Physiology*, **25**: 135-165.

Murashige, T., Skoog, F. 1962.A revised medium for rapid growth and bioassays with tobacco tissue cultures. *PlantPhysiology*, **115**: 493-497.

Narayana, K. S. 2008. Micropropagation of *Aloe vera.Project Report, School of Bio Engineering, S.R.M. University.*

Natali, L., Sanchez, I. C. and Cavallini, A. 1990**.***In vitro* culture of *Aloe barbadensis* Mill.: micropropagation from vegetative meristems. *Plant Cell, Tissue and Organ Culture,* **20**: 71-74.

Nayanakantha, N. M. C., Singh, B. R. and Gupta, A. K. 2010. Assessment of genetic diversity in *Aloe* germplasm accessions from India using RAPD and morphological markers. *Biological Science,***39**(1): 1- 9.

Nejatzadeh-Barandozi, F., Naghavi, M. R., Enferadi, S. T., Mousavi, A., Mostofi, Y. and Hassani, M. E., 2012. Genetic diversity of accessions of Iranian *Aloe vera*based on horticultural traits and RAPD markers. *Industrial Crops and Products,* **37**: 347–351.

Nooden, L. D. and Leopold, A. C. 1988. Senescence and aging in plants. *San Diego: Academic Press.* 1st edition.

Parmar, N., Kanwar, K. and Thakur, A. K. 2015. High efficiency plant regeneration from cotyledon explants of pomegranate (*Punica granatum* L.) cv. Kandhari Kabuli. *Vegetos,***28**(2): 160-165.

Payghamzadeh, K. and Kazemitadar, S. K. 2011.*In viro* propagation of Walnut- A review.*African Journal of biotechnology*, **10:** 290-311.

Prior*,* R. L. and Cao, G. 2000.Antioxidant phytochemicals in fruits and vegetables diet and health implications.*Horticulture Science*, **35**: 588-592.

Pugazhendhi, A. and Sekar, D. S. 2017. Successful explant response of *Aloe barbadensis* through micropropagation for the rapid regeneration of plants.*International Journal of Advance Research*, **5**(9): 80-86.

Rafiq, M., Dahot, M. U., Mangrio, S. M., Naqri, H. A. and Qarshi, I. A. 2012.*In vitro* clonal propagation and biochemical analysis of field established *Stevia rebaudiana* Bertoni. *Pakistan Journal of Botany*, **39**(7): 2467-2474.

Rathore, M. S., Chikara, J. and Shekhawat, N. S. 2011. Plantlets regeneration from callus cultures of selected genotype of *Aloe vera* L.- An ancient plant for modern herbal industries. *Applied Biochemistry and Biotechnology,***165** (7): 860-868.

Reid, J. B. and Howell, S. H. 1995.The functioning of hormones in plant growth and development.*Plant Hormones, Physiology, Biology, Molecular Biology,* **2:** 441-485.

Reynolds, T. and Dweck, A. C. 1999. Aloe vera leaf gel: A review update. *U. S. National Library of Medicine, National Institute of Health,* **68**(1-3): 3-37.

Richwine, A. M., Tipton, J. L. and Thompson, A. 1995. Establishment of *Aloe* Gasteria and Haworthia shoot cultures from inflorescence explants. *Horticulture Science,***30**:1443–1444.

Roy, S. C. and Sarkar A. 1991.*In vitro* regeneration and micropropagation of *Aloe vera* L. *Scientia Horticulturae*, **47**(1-2):

107-113.

Sabater, B. Hormonal regulation of senescence. 1985. *Hormonal Regulation of Plant Growth and Development India: Agro Botanical Publ*ications, **1**: 169-217.

Sahoo, S. and Rout, G. R. 2014. Plant regeneration from leaf explants of *Aloe barbadensis* Mill. and genetic fidelity assessment through DNA markers. *Physiology and Molecular Biology of Plants*, **20**(2): 235–240.

Sanatombi, K. and Sharma, G. J. 2008.*In vitro* propagation of *Capsicum chinense* Jacq.*Biologia Plantarum,* **52**(3): 517-520.

Sarkar, A. and Roy, S.C. 1984.Quick propagation of aloin-yielding plants through tissue culture.*Applied Biotechnology for Medicinal, Aromatic and TimberPlants,* pp.101-108.

Schween, G. and Schwenkel, H. G. 2003.Effect of genotype on callus induction, shoot regeneration and phenotypic stability of regenerataed plants in green house of *Primula ssp. Plant Cell Tissue and Organ Culture*, **72**: 53-61.

Shekhawat, M., Kannan, N., Manokari, M. and Ravindran, C. P. 2015.Enhanced micropropagation protocol of *Morinda citrifolia* L. through nodal explants.*Journal of Applied Research or Medicinal and Aromatic Plants,* **2**(4).

Shekhawat, N. S., Rathore, M. S., Shekhawat, S. Choudhary, S. K., Phulwaria, M., Manoj, H., Rai, K., Vibha, J. B., Nitika, Rathore, S., Patel, A. K. and Kataria, V. 2013. Micropropagation of *Aloe Vera* for improvement and enhanced productivity.*Climate Change and Plant Abiotic Stress Tolerance*, 1st Edition: 857-880.

Shukla, N, Rao, N. N. and Sharma, A. 2016.Micropropagation and elicitation studies in *Aloe vera. Asian Journal of Pharmaceutical and Clinical Research,* **9(1):** 54-60.

Singh, B. And Sood, N. 2009. Significance of explant preparation and sizing in *Aloe vera.* L. - A highly efficient method for *in vitro* multiple shoot induction. *Scientia Horticulturae,***122**: 146-151.

Singh, M., Rathore, M. S., Panwa, D., Rathore, J. S., Dagla, H. R. and Shekhawat, M. S. 2009. Micropropagation of selected genotype of *Aloe vera* L.: An ancient plant for modern industry. *Journal of Stationary Forestry*, **28**(8): 935-950.

Singh, N., Garg, A., Yadav, K. and Kumari, S. 2010. Influence of growth regulators on the explants of *Commiphora mukul* (Hook. ex Stocks) Engl. under *in vitro* conditions*. Researcher*, **2**(7): 41-48.

Skoog, F. and Miller, C. O. 1957. Chemical regulation of growth and organ formation in plant tissue cultured *in vitro*. *Symposium of Society of Experimental Biologist,***11**: 118-131.

Smith, M. L. A. 1994. Plant tissue culture.*Encylopedia of Agricultural Sciences, Academic Press,* **3**: 369-384.

Snedecor, G. W. and Cochran, W. G. 1972.Statistical method. *Lowa State University Press, Lowa*, **6:** 258-298.

Souza, V. and Lorenzi, H. 2005. Botanica sistematica: guia ilustrado para identificacxao das famılias de Angiospermas da flora brasileira, baseado em APG II. *Instituto Plantarum, Nova Odessa*, **640**.

Suhasini, S. C., Patil, S. N., Prabhuling, G., Venkateshalu and Jagadeesh, S. L. 2017.A suitable explant for culture establishment in pomegranate (*Punica granatum* L.).*Advances in Bioresearch*, **8** (5): 27-30.

Sujatha, M. and Mukta, N. 1996.Morphogenesis and plant regeneration from tissue cultures of *Jatropha curcas.Plant Cell Tissue and Organ Culture* ,**44**: 135-141.

Surafel, S., Gamachu, O. and Abel, D. 2018.*In vitro* propagation of *Aloe vera* Linn from shoot tip culture.*GSC Biological and Pharmaceutical Sciences*, **4**(2): 1-6.

Tamas, I. A., 1995. Hormonal regulation of apical dominance in plant hormones: Physiology, biochemistry and molecular biology, *Davies, P.J. (Ed.). Kluwer Academic Publishers, Dordrecht, Netherlands,* pp: 572-597.

Tanabe, M. J. and Horiuchi, K. 2006. *Aloe barbadensis* Mill.*ex vitro* autotrophic culture. *Journal ofHawaiian Pacific Agriulture,* **13**: 55-59.

Thorpe, T. A. 1990. The current status of plant tissue culture. Plant tissue culture: Applications and limitations. *Elsevier, Amstardam*, pp. 1-33.

Thorpe, T. A. 2000. History of plant tissue culture. In: (Smith, R.H., ed.) Plant tissue culture: *Techniques and Experiments. Academic Press*, London, pp. 2-32.

Tyagi, A. P., Comai, L. and Byers, B. 2001. Comparison of plant regeneration from root, shoot and leaf explants in pigeon pea (*Cajanus cajan*) cultivars. *SABRAO Journal of Breeding and Genetics*, **33**: 59-71.

Tyagi, R. K., and Prakash, S. S. 2004. For *in vitro* micropropagation and medium term conservation of jojaba.*Biologia Plantarum*, **48**: 119-123.

Vasil, I. K. 2002. The wandering of Botanist.*in vitro*. *Plant Cell Developmental Biology,***38**: 383-395.

Velcheva, M., Faltin, Z., Vandi, A., Eshdat, Y. and Perl, A. 2005.Regeneration of *Aloearborescens* via organogenesis from young inflorescences.*Plant Cell, Tissue and Organ Culture,* **83**: 293- 301.

Vesely, J., Havlicek, L. and Strnad, M. 1994. Inhibition of cyclin dependent kinases by purine analogues.*Europian Journal of Biochemi*stry, **224**: 771-786.

Wang, L., Zheng, S. X. and Gu- Z. J. 2002.*In vitro* culture of tetraploids of *Aloe vera* (L.).*Acta-Horticulturae Sinica,* **29** (2): 176-178.

Wenping, D., Shi, D., Xu, L., Yu, G., Mili, W. 2004. A preliminary study on the induction and propagation of adventitious buds for *Aloe vera* L. *South East China Journal of Agricultural Sciences,***17**(2): 224-227.

Wu, Y. X. and Xie, Q.H. 2002.Some influential factors for shoot organogenesis and plant regeneration in *Aloe vera. South West China Journal of Agricultural Sciences*, **15**: 190-192.

Xiaohong, L., and Jun, L., 2010.Biological research advancement in *Aloe vera*.*Journal of Medicinal Plant Research*, **5**(7): 1046-1052.

Yadav, L. 2008. Standardization of micropropagation protocol in Ghritkumari (*Aloe barbadensis* Mill.). M.Sc. Thesis, Rajasthan Agricultural University, Bikaner.

Zhang, W., Lin, X., Takano, H., Takio, S. and Ono, K. 2004. Efficient plant regeneration from suspension cells of *Allium cepa* L. *Plant Cell Rep*orts, **23**: 371-376.

Zhou, G., Ding, H., Shi, W. and Cheng, L. 1999.Fast asexual propagation of *Aloe vera*.*ScientiaHorticulturae*, **26**(6): 410-411.

Influence of Plant Growth Regulators, Genotypes and Explants on Micropropagation of Gwarpatha [*Aloe vera* (L.) Burm.]

Divya Dixit* (Investigator)

Dr. M. L. Jakhar** (Major advisor)

ABSTRACT

Present investigation was carried out with the objectives to determine the optimum level of different plant growth regulators for rapid shoot multiplication of *Aloe vera,* to study the effects of genotypes and explants on micropropagation of *Aloe vera,* to standardize protocol for rooting and hardening of micropropagated plantlets.

Auxiliary shoot explants were inoculated on MS medium supplemented with various concentrations of cytokinin (BAP 0.5 – 6.0) and BAP (0.5-6.0 mg/l) and auxin (NAA 0.1 – 0.6) in combination for direct shoot bud proliferation. For rooting, auxins (NAA/IBA) were used at the concentrations of 0.5-2.0 mg/l singly and 0.5-2.0 mg/l NAA with 0.5 and 2.0 mg/l IBA in combinations. The cultures were incubated at 25 ± 2°C under 14:10 hours photoperiod with a light intensity of 3000 lux. The explants induced multiple shoots along with either callus or root or both under the influence of plant growth regulators.

Maximum shoot bud induction was obtained when MS medium supplemented with 4.0 mg/l BAP and 4.5 mg/l BAP + 0.6 mg/l NAA. Maximum rooting was induced at 1.5 mg/l IBA and 0.5 mg/l IBA + 2.0 mg/l NAA. These most responsive levels of plant growth regulators were used to illustrate the effect of explants (auxiliary and apical shoot) and genotypes (JA-1, JA-2 and JA-3) and the results showed significant differences for shoot proliferation and rooting among both explants and genotypes. Auxiliary shoot explant was found better for both shoot proliferation and root induction. Genotype JA-1 was better for shoot proliferation and JA-2 was more responsive for profuse rooting. The rooted plantlets showed 80 per cent survival in culture room and 74 per cent in shade house during the process of hardening.

---

* A post graduate student, Department of Plant Breeding and Genetics, S. K. N. College of Agriculture, Jobner. Sri Karan Narendra Agriculture University, Jobner.

** Thesis submitted in partial fulfillment of the requirement for M.Sc. (Ag.) degree in Plant Breeding and Genetics under the supervision of Dr. M. L. Jakhar, Professor, Department of Plant Breeding and Genetics, Sri Karan Narendra Agriculture University, Jobner.

ग्वारपाठा (*एलो वेरा* (एल.) बर्म) के सूक्ष्म प्रवर्धन पर पादप वृद्धि नियामकों, जीन प्रारूपों एवं कर्तोतकों का प्रभाव

दिव्या दीक्षित*
(शोधकर्ता)

डॉ. एम.एल. जाखड़**
(मुख्य सलाहकार)

अनुक्षेपण

यह अध्ययन एलो वेरा के तीव्र बहुगुणन के लिए विभिन्न पादप वृद्वि नियामकों का इष्टतम् स्तर निर्धारित करने, जीन प्रारूपों एवं कर्तोतकों के *एलो वेरा* के सूक्ष्म प्रवर्धन पर प्रभाव का अध्ययन करने एवं प्रसारित पादपों में जड़ प्रेरण तथा कठोरता के उद्देश्य से किया गया।

पार्श्व प्ररोह कर्तोतकों को प्ररोह प्रसरण के लिए एम एस माध्यम पर साइटोकाइनिन (बी.ए.पी. 0.5–6.0 मिग्रा/ली.) एव बी.ए.पी. (0.5–6.0 मि.ग्रा./ली.) और ऑक्सिन (एन.ए.ए. 0.1 से 0.6 मि.ग्रा./ली.) के संयोजन युक्त सान्द्रताओं के साथ संचरित किया गया। जड़ प्रेरण हेतु आक्सिन्स (एन.ए.ए./आई.बी.ए.) का उपयोग 0.5 –2.0 मि.ग्रा./ली. और 0.5 से 2.0 मि.ग्रा. /लीटर एन.ए.ए. के साथ 0.5 और 2.0 मि.ग्रा. /ली. आई.बी.ए. के संयोजन में किया गया। सभी संवर्धन 25 $\pm$ 2 डिग्री सेल्यिसस तापमान पर 3000 लक्स पर 14:10 घण्टे प्रकाश अवधि पर सेंचित किए गए। पादप वृद्वि नियामकों के प्रभाव में कर्तातकों ने या तो कैलस या जड़ या दोनों के साथ प्ररोह–प्रसरण प्रदर्शित किए।

अधिकतम प्ररोह कली प्रसरण 4.0 मि.ग्रा./ली. बी.ए.पी. एवं 4.5 मि.ग्रा./ली. बी.ए.पी. + 0.6 मि.ग्रा./ली. एन.ए.ए. के साथ संचरित माध्यम पर प्राप्त किया गया। अधिकतम जड़ प्रेरण 1.5 मि.ग्रा./ली. आई.बी.ए. और 0.5 मि.ग्रा./ली. आई.बी.ए. + 2.0 मि.ग्रा./ली. एन.ए.ए. पर देखा गया। पादप वृद्वि नियामकों के इन सर्वाधिक उत्तरदायी सान्द्रता स्तरों का उपयोग कर्तोतकों (पार्श्व एवं शीर्षस्थ प्ररोह) और जीन प्रारूपों (जे.ए.–1, जे.ए.–2 एवं जे.ए.–3) के प्रभाव को चित्रित करने हेतु किया गया एवं परिणामों में सार्थक अन्तर देखा गया। पार्श्व प्ररोह कर्तोतक को प्ररोह प्रसरण एवं जड़ प्रेरण दोनों के लिए श्रेष्ठतर पाया गया। जीन प्रारूप जे.ए.. –1 को प्ररोह प्रसरण जबकि जे.ए.–2 को विपुल जड़ प्रेरण के लिए श्रेष्ठतर पाया गया। जड़ प्रेरित पादपकों की कठोरता की प्रक्रिया के दौरान पादपक संवर्धन कक्ष में 80 प्रतिशत तथा छाया गृह में 74 प्रतिशत जीवित रहे।

---

*कृषि स्नातकोत्तर छात्रा, पादप प्रजनन एवं आनुवांशिकी विभाग, श्री कर्ण नरेन्द्र कृषि महाविद्यालय, जोबनेर (श्रीकर्ण नरेन्द्र कृषि विश्वविद्यालय, जोबनेर),

**कृषि में स्नातकोत्तर उपाधि प्राप्ति की आंशिक आवश्यकता की पूर्ति के लिये वर्तमान शोधकार्य डॉ. एम. एल. जाखड, आचार्य, पादप प्रजनन एवं आनुवांशिकी विभाग, श्री कर्ण नरेन्द्र कृषि महाविद्यालय, जोबनेर (श्री कर्ण नरेन्द्र कृषि विश्वविद्यालय, जोबनेर) के निर्देशन में प्रस्तुत किया गया।