“IN VITRO REGENERATION OF ALOE VERA
(Aloe barbadensis Mill)"

M.Sc. (Ag.) Thesis

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in

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CERTIFICATE - I

This is to certify that the thesis entitled "In vitro regeneration of aloe vera (Aloe barbadensis Mill)" submitted in partial fulfillment of the requirements for the degree of Master of Science in Agriculture of the Indira Gandhi Krishi Vishwavidyalaya, Raipur, is a record of the bonafide research work carried out by Shashi Kiran under my guidance and supervision. The subject of the thesis has been approved by the Student's Advisory Committee and the Director of Instructions.

No part of the thesis has been submitted for any other degree or diploma or has been published/published part has been fully acknowledged. All the assistance and help received during the course of the investigations have been duly acknowledged by her.

Dr. Alice Tirkey

Date: 17/10/2017
(Major Advisor and Chairman)

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Member: Dr. Arti Guhey
CERTIFICATE - II

This is to certify that the thesis entitled "In vitro regeneration of aloe vera (Aloe barbadensis Mill)" submitted by Shashi Kiran to the Indira Gandhi Krishi Vishwavidyalaya, Raipur, in partial fulfillment of the requirements for the degree of Master of Science in Agriculture in the Department of Genetics and Plant Breeding has been approved by the external examiner and Student's Advisory Committee after oral examination.

Date: 13-12-2017

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Major Advisor

Head of the Department

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Approved/Not approved

Director of Instructions
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Shashi Kiran
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## LIST OF SYMBOLS & ABBREVIATIONS

| SYMBOLS/ABBREVIATIONS | DESCRIPTION                                                      |
|-----------------------|------------------------------------------------------------------|
| %                     | Per cent                                                         |
| &                     | And                                                              |
| BAP                   | Benzyl amino purine                                              |
| BA                    | Benzyladenine                                                    |
| Fig.                  | Figure                                                           |
| i.e.                  | That is                                                          |
| IAA                   | Indole acetic acid                                               |
| IBA                   | Indole butyric acid                                              |
| KNO₃(NH₄)₂SO₄          | Potassium nitrate ammonium sulphate                              |
| mm                    | Millimeter                                                       |
| NAA                   | Naphthalene acetic acid                                          |
| μg                    | Microgram                                                        |
| μl                    | Microlitre                                                       |
| μm                    | Micromolar                                                       |
| MI                    | Millilitre                                                       |
| M                     | Molar                                                            |
| Min                   | Minute                                                           |
| MS                    | Murashige and Skoog                                              |
| Ng                    | Nanogram                                                         |
| viz.                  | Namely                                                           |
| et al.                | Co-workers                                                       |
| WRC                   | White’s root culture                                             |
THESIS ABSTRACT

Title of Thesis  "In vitro regeneration of aloe vera (Aloe barbadensis Mill)"
Full name of Student  Shashi Kiran
Major Subject  Genetics and Plant Breeding
Name and Address of Major Advisor  Dr. Alice Tirkey, Scientist, Department of Genetics and Plant Breeding, IGKV, Raipur, C.G.
Degree to be awarded  Master of Science (Ag.) in Genetics and Plant Breeding
Signature of Major Advisor
Date - 17/10/17
Signature of Student

ABSTRACT

The present investigation entitled "In vitro regeneration of aloe vera (Aloe barbadensis Mill)" was carried out at Herbal science garden of college of agriculture IGKV, Raipur during summer season 2016-17. The experiment was conducted in randomized block design with 13 accession collected from three agro-climatic zones of C.G and were evaluated for estimation of genetic variability, coefficient of correlation, path analysis and cluster analysis.

Analysis of variance revealed significant differences among the aloe vera genotype for the character. Direct selection for plant height (cm), leaf length (cm), leaf width (cm), thickness of leaf (cm), no. of spines/leaf, no. of leaf/plant, may be advantageous for selection the high leaf gel content (g) accessions in aloe vera from the available gene pool. High heritability coupled with high genetic advance as percentage of mean was found in the plant height (cm), leaf length (cm), no. of spines/leaf, no. of suckers, gel content/leaf (g). The D^2 analysis for inter and intra cluster distance among the four cluster showed that maximum inter cluster distance was observed in between I and IV(5.350) followed by cluster IV and
This suggested that the hybridization programme involving parents from these cluster is expected to give higher frequency of better segregates or desirable combination for development of useful genetic stocks or varieties. The minimum inter cluster distance was observed in between II and III (2.667) followed by cluster I and II (2.883). The maximum intra cluster distance was observed in cluster IV (2.183) followed by cluster I (1.298), cluster II (1.5), cluster III (1.03). Correlation study indicated that gel content/plant(g) had positive and significant with plant height(cm), leaf length (cm), leaf width (cm), thickness of leaf, no. of spines/leaf. Path coefficient analysis showed that gel content/plant (g) had the highest positive direct effect with leaf length (cm) followed by leaf width (cm) followed by thickness of leaf followed by no. of spines/leaf and highest negative direct effect is plant height(cm), no of suckers/plant.

In vitro regeneration of aloe vera (Aloe barbadensis Mill) was carried out in commercial tissue culture laboratory, IGKV, Raipur, CG. The apical shoot bud as an explant was found best in, in vitro culture in present study. The concentration and combinations of hormones which showed highest response for shoot initiation was 2mg/l BAP+0.2 NAA. No. of explant shoot is 13 initiated in this media. Initiation media was 2mg/l BAP+0.2 NAA. Multiplication media is 1.5mg/l BAP+0.2NAA. 6 generation shoot multiplied. The concentration and combinations of hormones which showed high response for root initiation was MS media ( half ) + 0.5 mg/l IBA + 500mg/l activated charcoal. Regenerated plantlets of 6-8 weeks old with well developed roots were transferred to sterilized cocopit in poly house. After 1 week transferred to sterilized earthen pots containing a mixture of sand, FYM, soil at a ratio of 1:2:1. In vitro regenerated plantlets were hardened and successfully established in field.
शोध सारांश

शोध का शीर्षक - एलो बारबादेसिस में इन बिट्र्यो पुनर्जनन

विद्वानों का पूरा नाम - शशि किरण

मुख्य विषय - अनुवादितक एवं पादप प्रजनन

मुख्य संलग्नकर का नाम - खूं एलिसा रिक्स वैज्ञानिक अनुवादितक एवं पादप प्रजनन

प्रदान की जाने वाली उपाधि - एम.एस. आर. कृष्ण अनुवादितक एवं पादप प्रजनन

मुख्य संलग्नकर के हस्ताक्षर

विद्वानों के हस्ताक्षर

दिनांक - 12/10/17

सारांश

कार्यालय शोध शीर्षक "एलो बेसा में इन बिट्र्यो पुनर्जनन (एलो बारबादेसिस मिल")"

उच्च जेल मामले के लिए एलो बेसा जनसंख्या की स्कूलिंग गर्मी में 2016-17 के दौरान आई जी के सी. सामूहिक साइट युग्म में क्रिया गया था। यह वार्ता व्यक्तिक सिद्धांत में क्रिया गया था, जिसमें अनुवादितक परिवर्तन-गतिविधियाँ, सहकर्मी, पथ विद्यालय, और समूह विद्यालय के आक्रान्त के लिए 13 एक्सीजन का उपयोग किया गया।

विभिन्न क्रांति का वितरण किया, फौज की उंचाई (सेना.) के लिए प्रवक्ता घरेलू के लिए एलो बेसा जीनोटाइप के बीच पहले पुर्ता अंतर वह पता चला पत्ति की लंबाई (सेना.) फौज की उंचाई (सेना.) पत्ति की दूरी (सेना.) पत्ति के कार्यों के संरचना प्रश्नों की संख्या से एलो बेसा में उच्च जेल मामला उपयोग के लिए जीनोटाइप का उपयोग लाभार्थी ने सक्षम है। उच्च अनुवादितक के साथ उच्च अनुवादितक आग्रह माध्यम प्रतिरोध में पीएच की उंचाई (सेना.), पत्ति की लंबाई
जीनोटाइप का चयन लाभकारी हो सकता है। उच्च आनुवाषिकता के साथ उच्च आनुवाषिक आग्रिम माध्य प्रतिशत में पौधे की उंचाई (सेमी.), पत्ती की लम्बाई (सेमी.), कांटों की संख्या, प्रत्येक पत्ती, अंत: भूस्तारी की संख्या, जोल मात्रा प्रति पत्ती (ग्रा.) चार क्लस्टर के बीच अंतर और अंतर क्लस्टर प्रतिशत है, अधिकतम अंतर क्लस्टर दूरी I और IV (5.350) के बीच देखा गया था, इसके बाद क्लस्टर IV और III (3.569) ने यह सुझाव दिया कि संकरण कार्यक्रम शामिल है, इन क्लस्टर से जनक को उपयोगी आनुवाषिक स्ट्रॉक्स किया के विकास के लिए बेहतर या वाच्छनीय संयोजन का उच्च आयुक्त है। न्यूनतम अंतर क्लस्टर दूरी के दिल्लीय और तृतीय के बीच (2.667) तथा क्लस्टर I और III के बीच 2.883 दूरी देखा गया। अधिकतम अंतर क्लस्टर दूरी क्लस्टर IV (2.183), क्लस्टर I (2.183), क्लस्टर II (1.5), क्लस्टर III (1.03)। सहसंबंध अध्ययन से संकेत मिलता है कि प्रत्येक पत्ती जोल मात्रा में धनालक महाशूरण के साथ पौधे की उंचाई (सेमी.), पत्ती की लम्बाई (सेमी.), पत्ती की चौड़ाई (सेमी.), पत्ती की मोटाई (सेमी.), कांटों की संख्या प्रति पत्ती है। पथ गुणाक विकल्पण से पता चलता है कि जोल मात्रा (ग्रा.) से पत्ती की चौड़ाई (सेमी.), के बाद पत्ती की लम्बाई (सेमी.), के साथ सबसे अधिक सकारात्मक प्रयोक्त्र प्रभाव था, जिसके बाद पत्ती की मोटाई (सेमी.), कांटों की संख्या तथा पौधे की उंचाई (सेमी.), अंत: भूस्तारी की संख्या में उच्चतम नकारात्मक सिद्धा प्रभाव था।

एलो बेसा (एलो बासबाड़ीस का) के इन विद्यो पुनर्जनन को वाणिज्यिक उत्तर संचरण प्रयोगशाला, आईजीकेएच, रायपुर छातीसगढ में किया गया। शीर्षस्थ शाखा कलिका (Apical shoot bud) अच्छे अन्वेषक के रूप में पुनर्जनन के अध्ययन में पाया गया। 2 मिली. ग्रा./ बी ए पी (BAP) + 0.2 एन ए ए (NAA) हार्मोन सांदर्भ संयोजन की मात्रा शाखा प्रारंभ में सबसे अधिक प्रतिक्रिया दिखाते हैं। एन एस (MS) (आधा) + 05 मिली. ग्रा. / आई बी ए (IBA) + 500 मिली. ग्रा./ली संक्रिया चारकोल सांदर्भ संयोजन की मात्रा जड़ों के विकास के लिए सबसे अधिक प्रतिक्रिया दिखाते हैं। पौधे को जीवाणुहीन कोकोपिट में स्थानांतरित करते हैं। एक सप्ताह बाद जीवाणुहीन पालिक्षेप में रेत, एक बाई एम (FYM) गोबर की खाद : मिटटी 1:2:1 के अनुपात मं स्थानांतरित किए। इन विद्यो पुनर्जनन के द्वारा पौधे को सफलतापूर्वक क्षेत्र (Field) में स्थापित किया गया।
CHAPTER-I
INTRODUCTION

_Aloe barbadensis_ Mill. belongs to the family Liliaceae (Anonymous, 1985). Aloe vera (Indian Aloe) is an important medicinal and miracle plant. The plant prefers sunny weather, requires well-drained soil and can grow in nutritionally poor soil. Pharmaceutical and cosmetic industry has great demand for Aloe vera. In Chhattisgarh total area under aloe vera cultivation was 333 ha. in C.G with 3592 metric tonnes production in 2015-16 (Directorate of Horticulture, C.G). Its therapeutic use was reported earlier by several scientists (Cera _et al._, 1980; Afzal _et al._, 1991; Davis _et al._, 1988).

It is commonly called as 'Burn plant'. It is a xerophyte and can be grown even in dry lands under rain fed conditions. _Aloe_ is a coarse looking perennial plant with a short stem, found in the semi-wild state in many parts of the country. Leaves 30-60 cm long, erect, crowded in a basal rosette, full of juice, glaucous-green, narrow lanceolate, long-acuminate, smooth except for the spiny teeth on the margins. Scape longer than leaves, scaly, branched. Flowers yellow, in dense racemes terminating the scapes. Commercial _Aloes_ are obtained from wild as well as cultivated plants. Propagation is primarily by means of suckers (or) offshoots, which are separated carefully from mature plants and transplanted. Medium sized suckers are chosen and carefully dugout without damaging the parent plant at the base and can be directly planted in the field. Plants will produce a commercial crop in one year.

The plant has stiff gray-green lance-shaped leaves containing clear gel in a central mucilaginous pulp. Aloe vera has been used for several thousands of years in folk medicine in many cultures from ancient Egypt, Greece, and Rome to China and India (Kemper and Chiou 1999). Aloe vera gel obtained from leaf pulp has been reported to have multiple beneficial properties for wound healing, including the abilities to penetrate and anesthetise tissues, arrests bacterial, fungal and viral growth, acts as an anti inflammatory agent and enhances blood flow (Klein and Penneys 1988, Haller 1990, Davis _et al._, 1994, Heggers _et al._, 1996, Yao _et al._, 2009, Sahu _et al._, 2013) Medicinal properties are due to aloin, isobarbadonin and
emodin (Chopra et al., 1956, Singh and Sood, 2009) and fleshy leaves of Aloe have been found to contain about 200 bioactive constituents. (Waller et al., 1978; Vogler and Ernest 1999). The use of aloe in therapeutic is reported by several scientists (Cera et al., 1980; Afzal et al., 1991; Davis et al., 1988). A gel in the leaves makes an excellent treatment for wounds, burns and other skin disorders, placing a protective coat over the affected area, speeding up the rate of healing and reducing the risk of infection. (Ahmed et al., 2007). Aloe vera is propagated through lateral buds, which is slow, very expensive and low income practice (Meyer and Staden, 1991).

Aloe vera gel obtained from leaf pulp has been reported to have multiple beneficial properties for wound healing, including the abilities to penetrate and anesthetise tissues, arrests bacterial, fungal and viral growth, acts as an anti inflammatory agent and enhances blood flow (Klein and Penneys 1988, Haller 1990, Davis et al., 1994, Heggers et al., 1996, Yao et al., 2009, Sahu et al., 2013) Medicinal properties are due to aloin, isobarbadonin and emodin (Chopra et al., 1956, Singh and Sood, 2009) and fleshy leaves of Aloe have been found to contain about 200 bioactive constituents (Waller et al., 1978, Vogler and Ernest 1999). Aloe vera has been cultured in vitro by various researchers (Natali et al., 1990; Roy and Sarkar, 1991; Abrie and Staden, 2001). Aloe vera propagated mainly through vegetative means, as due to mail sterility, sexual reproduction was ineffective. Propagation is primarily by means of sucker or off shoots, which are separated carefully from mature plants and transplanted. Although Aloe vera propagates vegetatively in it’s natural state, but the process is too slow (Meyer and Staden, 1991). Aloe vera propagates vegetatively in its natural state. However, propagation rate is very slow because a single plant can produce only three to four lateral shoots in a year. Moreover, the production of Aloe leaves is insufficient to meet the industry demand in India (Aggarwal and Barna, 2004) and the production of cosmetics, foods and pharmaceuticals containing aloe vera has experienced a slow increase due to limited availability of raw material with high quality (Campestrin et al., 2006).
The technique to tissue and organ culture is used for rapid multiplication of plants, for genetic improvement of crops, for obtaining disease-free clones and for progressive valuable germplasm (Bhojwani & Razdan, 1992).

Little information is available about the genetic diversity inter and intra specific variety and genetic relation among aloe vera. Therefore attempts are made to analysis possible untapped genetic diversity which is extremely essential for the section and improvement of aleo vera genetic parameter of variation and character association provide information about expected response of various character and helps in developing suitable breeding processor for their improvement. Nature and magnitude of variability in existing plant materials and association among various characters are per requisites for yield and selection of better plant site. Path coefficient analysis permits further facilities important trait identified. This parameter however varies with type of materials use of the environment materials to which the accessions are subjected. Such studies have not been made yet in state of Chhattisgarh. Keeping the above fact, the present investigation was carried out to study the following objectives:

1. Screening of Aloe vera germplasm for high gel content.
2. To standardize protocol for high frequency in vitro regeneration of Aloe vera using various explants.
3. To study the effect of different combinations and concentration of plant growth regulators on large scale multiple shooting and establishment of plantlet
During past few years, there has been an increase interest for \textit{in vitro} multiplications and germplasm conservation of rare, endangered, aromatic and medicinal plants. Mass propagation of plant species through \textit{in vitro} culture is one of the best and most successful examples of commercial application of plant tissue culture technology. Recently, there has been much progress in this technology for some plant which has multiple economic values and become very attractive due to its energy resources.

Micropropagation is the practice of rapidly multiplying 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. It is also used 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. Micropropagation is a set of procedures that multiply plants in tissue culture with minimal genetic and epigenetic variability. Micropropagation is one of the innovative methods of asexual propagation, which proved to be effective for in vitro propagation of medicinal and endangered plants. Clonal propagation through tissue culture has the potential to provide high multiplication rates of uniform genotypes, resulting in short term gains. Cloning allows for the immediate and total capture of genetic gain. Clonal propagation through tissue culture popularly called micropropagation can be achieved in short time and space. Micropropagation technique would play an important role in mass propagation of elite genotypes, conservation of germplasm, genetic improvement of plants and production of pharmaceuticals yet it not replaced traditional methods of propagation, but has found its own niche in areas where it is clearly superior. Micropropagation involves following methods:

1. Establishment.
2. Multiplication.
3. Rooting.
4. Hardening.

*Aloe barbadensis*, one of the world’s most widely used substances for medicine, cosmetics and food, is vegetatively propagated using the suckers. This conventional method is very slow and could not meet the required number of planting materials needed to establish a large-scale plantation. An alternative propagation method is by tissue culture (Eufrocinio and Malasa, 2005).

Aloes have thick, tapered, spiny leaves grow from a short stalk near ground level and grow from the base in the rosette pattern. Mature plants can grow as tall as 2½ to 4 feet with the average being around 28 to 36 inches in length. Each plant usually has 12 to 16 leaves that, when mature, may weigh up to three pounds. The plants can be harvested every 6 to 8 weeks by removing 3 to 4 leaves per plant (IASC, 2002). Aloes display unusual patterns of variation among populations and species and inconsistent integration among them. The morphology of leaves, flowers and inflorescence varies widely and inconsistently. Latter new concepts and classification systems have been adopted to solve this classification problem. Besides gross morphology, other characters such as pollen, chemistry and isoenzyme have been employed by taxonomists to reveal the relationships in the genus (Dessalegn, 2006). A more generalized botanical distinction among the *Aloes* is achieved by observing the trunk and leaves. In this way, three large groups of *Aloes* can be distinguished: acauleas (without a trunk), subcauleas (visible trunk but with a reduced size) and cauleas (having a large and branched trunk) (Bassetti and Sala, 2005; Dessalegn, 2006).

### 2.1. Diversity, Distribution and Ecology of Aloe vera

There are many species of *Aloe* grown throughout the world. However, only few species are grown commercially today, with *Aloe barbadensis* Miller and *Aloe aborescens* being the most popular (IASC, 2002). In Africa, *Aloe* occurs over much of Sub-Saharan Africa, although they are mainly concentrated in the southern and eastern regions of the continent and only two or three species are found in western Africa. Some species of aloe vera are widespread, while some
occupy a single locality. Many countries have the endemic species— the highest rate of endemism being in Madagascar and isolated Islands of Indian Ocean, Mauritius, South Africa and a large number of endemic taxa also exist in Tropical East Africa. Conversely it is not surprising that very small countries such as Burundi and Rawanda have no endemic species (Dessalegn, 2006).

The habitat occupied by *Aloe* varies from forest, wooded-grasslands and wood lands. Several species occur on expanses of rocks, rooted into soil pocket or cervices. Others are also found in mountains, cliff faces and beaches and even under the spray of waterfalls. Most aloes have succulent xerophytic leaves, adapted to survive in areas of low or erratic precipitation. The fleshy leaves with sunken stomata and thick cuticular wax help them to resist drought. Hence *Aloe* can be regarded typical to semi-arid and dry deserts. Sharp thorns, spines, and usually bitter leaf sap, are good deterrents for many herbivores. Generally speaking, *Aloe* grows in localities with annual rainfall ranges from as low as 350 to 400 mm to even as high as 1500 to 2000 mm (Ved et al., 2002; Dessalegn, 2006; Klopper and Smith, 2010). *Aloes* are keystone species in the ecosystem, being perennial plants, able to tolerate extreme environmental conditions and provide important source of shelter, nectar, food, and moisture especially for birds. It is thus imperative to document information on the biology, ecology, distribution and chemistry of these species in order to take appropriate measures to conserve and promote sustainable use of these botanical treasures (Demissew and Nordal, 2010).

### 2.2. Importance of *Aloe vera*

*Aloe* species have been known for their use in medicine, commerce and horticulture; thus become fascinating object of research in the chemical, pharmaceutical, economic and taxonomic fields. *Aloes* have multitude of uses as described below.

#### 2.2.1. Medicinal uses

Extracts of *Aloe* are complex mixture of a variety of biologically active components. *Aloe* extracts are usually used in treating many illnesses or symptoms. Historical accounts show that extracts of *Aloe* were mainly used as healing aids for tropical skin problems (Fujita, 2002). However, over the years, the use of the
extracts was changed from folk medicine applications to phytotherapeutics, in recent years. Gel of *Aloes* has anti-bacteria and anti-fungi effect (Agarry *et al*., 2005). Bassetti and Sala (2005) has reported that *Aloe* plant contains over 150 therapeutic active contents – including mucopolysaccharides, whose viscous or slimy capacity suggests its gastro-protective effect; anthraquinones with laxative action, restorative and repairing effects; and a wealth of vitamins and minerals. *Aloes* contain more potent stimulatory substances than any food in its own unique combination that boost immune system of patients, thus promote healing, cleanse and relieve inflammatory conditions. Internal and external uses of extracts of *Aloes* are known and claimed to mitigate or completely cure many different disease like abrasion, acne colds, hypertension, various infections, sunburns, dandruff, allergic reactions, insect bites, diabetes, arthritis, vaginitis, rashes, AIDS, tuberculosis, cancer, herps etc (Kemper and Chiou, 1999; Hedendal, 2002; Mascola *et al.* 2004). Methanol and water extract of *Aloe debrana* leaves have shown suppression against malaria in *Parasitaemia berghei* infected mice (Deressa *et al.* 2010). Nowadays, *Aloe* is widely used as beverages, cosmetics and ointments. Japan currently imports over fifty million dollars of *Aloe* per year to treat people with ulcers and digestive problems (Hedendal, 2002).

2.2.2. Ornamental uses

*Aloes* are also popular as home and garden plants in both tropical and temperate regions and have become important in the horticulture industry. *Aloes* are used in gardens as ornamental plants in many African countries (Newton, 2004). *A. ballyi* Reynolds and *A. barberae* Dyer are commonly grown in streets of Nairobi. They are also used as decorative element in homes and gardens, e.g. *A. brevifolia, A. descoingsii,* and *A. marlothii* (IASC, 2008). Smaller and slow-growing species are popular pot plants for enthusiasts in many temperate countries. In more modern times the hollow dead stems of *Aloe* is cut into pieces to make various decorative items such as ash trays and other small containers (Dessalegn, 2006). It was also described by Todaro in Hortus Botanicus Panormitanus in 1878. *A. percrassa* described from Tigray and Gondar (Ethiopia) and Eritrea and *A. debrana* described from west of Debre Birhan, Shewa are closely related - they are herbaceous, acaulescent or semicaulescent and rosette-
leaved – thus belong to *A. percrassa* complex. *A. percrassa* (in the strict sense) has relatively few branched inflorescences, very rarely with second order branching.

*A. debrana*, on the other hand, has inflorescences nearly always with second order branching (Dessalegn, 2006). *A. percrassa* is distinguished from the rest of the groups by the large bracts which are 10-16 (-20) mm long. *A. percrassa* has leaves with crowded and slightly glaucous, inflorescences with just one level of branching 0–80 cm high with 5–12 racemes and brown color of old leaves when drying (Demissew and Nordal, 2010). The authors have documented that *A. percrassa* grows in sparsely vegetated rocky slopes and outcrops between 2100 and 2700 m.a.s.l. in Tigray and Gonder regions in Ethiopia and also in Eritrea. It is so far not known anywhere else. The main flowering period is from September to October, sometimes also in March to April *A. percrassa* is succulent herb, suckering from base to form small groups, mostly stem less, with crowded leaves, endemic with relatively wide geographical range in northern Ethiopia and Eritrea, occurring in mountain slopes 38 to 41 km North of Mayichew (78 to 81 km South of Mekelle), on Ambalage, 60 km from Mekеле to Adigrat and 20 km West of Adigrat towards Adwa together with *A. Adigratana* in Tigray floristic region. It is also found 20 km from Debak on Gondar to axun road in Amhara Floristic region and Quahtito plateau and Saganeiti in Eritrea (Dessalegn, 2006; Demissew & Nordal, 2010). Some studies revealed the existence of major threats to *Aloes* in Ethiopia due to destruction of natural habitats for agriculture, massive and extensive road construction, and urbanization (Dessalegn, 2006).

### 2.3. Tissue culture studies of *Aloe vera*

#### 2.3.1. Explants and sterilization of *Aloe vera*

Explants of *Aloes* are treated with different chemicals for sterilization. Many of the sterilization activities involve washing of the explants with clean water, treating them with some fungicidal and bactericidal chemicals, and rinsing the treated explants with sterile water. Choudhary *et al.*, (2011) collected fresh, healthy, and disease free explants and put them in beaker filled with water. The explants were, then, washed thoroughly with running tap water for 45 minutes, treated with 1% bavistin for 30 minutes, rinsed for 3 to 4 times with tap water and
2 to 3 times with distilled water to remove traces of fungicide remain, treated with 0.1% (w/v) mercury chloride solution for 20 minutes, and washed 5 times with autoclaved distilled water to remove traces of mercury chloride. Other sterilization procedures using different chemicals are reported (Hashemabadi and Kaviani, 2008; Singh et al., 2009). Injury on the explants of Aloe depends on the type of disinfection processes and the nature of the plant material. An important consideration in Aloe explants sterilization is that some species may have low lignin, thus may be easily damaged. For example, Aloe vera tissue has low lignin content and is very sensitive to disinfectants (Oliveira et al., 2009).

Utilizing an accurate sterilization procedure in tissue culture techniques can save time and energy. However, the explants must be sterilized and viable while sterilizing. This study aims at providing a new method to replace mercuric chloride. In this study sodium hypochlorite (commercial brand Clorox) with some drops of Tween80 10%, 15%, 20%, 25% and 30% were used. Applying a Kruskal-Wallis test (a nonparametric test), revealed that 5% sodium hypochlorite with 20 minutes of hard and constant shaking, gives the highest number (91.7%) of viable and sterilized explants with regeneration potential in Murashige and Skoog medium supplemented with IBA and TDZ and Zeatin. Using Pearson Chi square test (X=37.144, P value = 0.0001), the results revealed a significant relationship between the sodium hypochlorite concentration and percentage of surviving explants in Aloe vera barbadensis Mill. (Sanghi, 2015).

2.3.2. Micropropagation of Aloe vera

One of the major applications of plant tissue culture is micropropagation or rapid multiplication in limited time and space. The intervention of biotechnology or to be precise, plant tissue culture for accelerating clonal multiplication of desired clones and strains (high yielding) of medicinal, ornamental, basic food, industrial and commercial valuable plants through micro propagation and their conservation through establishing Tissue Banks or Gene Banks are warranted in the right earnest. Tissue culture and micropropagation of different species of Aloe as reported in the works of many researchers, yielded excellent results (Baksha et al., 2005; Hashemabadi and Kaviani, 2008; Garro-Monge et al., 2008; Bhandari et al., 2010; Abdi et al., 2013). The accumulation of phenolic compounds in the
media is the biggest problem. Pre-washing of the tissue with Polyvinylpyrrolidone (PVP) removed phenolic compounds secreted from excised explant surfaces to aid in the adaptation of the explants in the new media for second growth. But adventitious roots were not induced when PVP was directly supplemented on root induction media. Addition of PVP in initial root induction media resulted in absorption of phenolic compounds that might negatively affect the initial induction of adventitious roots in *Aloe vera* (Lee *et al.*, 2011).

### 2.3.3. Roles of media, plant growth regulators and culture conditions

*In-vitro* culture is used for commercial production and is achieved in aseptic condition using different concentration and combination of plant growth regulators (PGRs). The concentration and combination of auxins and cytokinins in the nutrient MS medium are usually a key factor which determines success of plant regeneration. Generally speaking, high cytokinins-low auxin induces shoot bud formation, high auxin-low cytokinin induces root formation and when concentrations of auxin and cytokinin are comparable, callusing becomes predominant.

Nodal explants of *A. barbadensis* were placed in Murashige and Skoog (MS) medium containing different levels of kinetin or 6-benzylaminopurine (BAP) to induce multiple shoot formation. The best treatment for multiple shoot induction was 1 mg L\(^{-1}\) BAP, which produced an average of 11 shoots per explant in 1 mo. Individual shoots from the multiple shoot clumps were taken and transferred in MS medium containing different levels of indole-3-acetic acid (IAA), indole-3-butyric acid (IBA) or \(\alpha\)-naphthalene acetic acid (NAA) for rooting. The best auxin for root formation was NAA, with an optimum concentration of 0.10 mg L\(^{-1}\). Rooted plantlets were successfully transferred in the field. (Eufrocinio and Malasa, 2005).

Tanabe and Horiuchi (2006) reported that media containing BA and sucrose promoted shoot growth. A medium combination with 2 g/L sucrose + 2.2 \(\mu\)M BA showed regeneration of a new shoot in 80% of its cultures in 6 weeks. Exclusion of BA or sucrose resulted in regeneration in 40% or less of the cultures. Although BA plays an important role in shoot initiation. Field plants were used.

The most common PGRs used in the tissue culture and micropropagation includes auxin NAA and cytokinin BAP (Choudhary *et al.*, 2011). The type and
concentration of PGRs, added to basal or half strength MS and/or other growth media affect regeneration of plants. The commonest PGRs 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, pyrogallol, phloroglucinol and L-cysteine (to reduce excessive browning of explants) and adsorbents like activated charcoal and PVP (for checking excessive browning). And, yet, regeneration responses of explants of various plants are affected by pH, photoperiod, light intensity, RH, and temperature (Choudhary et al., 2011; Hashemabadi and Kaviani, 2008; Hosseini & Parsa, 2007; Narayana, 2008).

Adelberg et al. (2012) studied on agar and liquid medium at varied benzyladenine (BA) and meta-topolin (MT) concentrations (0, 1, 3.2, and 10 μM) for three successive culture cycles and then transferred to a greenhouse for growth. MT induced multiplication at the highest concentration (10 μM) and BA produced the greatest number of plantlets (at 3.2 μM) with optimal multiplication at approximately 6 μM. Cytokinin carryover reduced rooting from 92% (control) to 68% with either the 3.2 μM MT or 10 μM BA and at 10 μM MT only about 20% of the aloe plants rooted. Using liquid medium led to larger plants and lessened the cytokinin carryover effect on rooting without affecting the multiplication rate. Approximately 6 μM BA in liquid medium would be optimal for multiplication and rooting of A. barbadensis. Plants purchased from Altman Plants (Vista, CA), was used in this study.

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 2.5mg/L. Optimum growth of callus was achieved from RSL and LS explants on medium containing NAA+BAP+IBA(2.5+2+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. Specimens of Aloe vera were taken for explants preparation from garden of University Department of Botany.
2.3.4. Effect of different explants, shoot initiation and proliferation

Various concentration and combinations of BAP and kinetin in combination with IBA and NAA are commonly used in shoot initiation and proliferation of explants. Bhandari et al. (2010) reported that initiations of shoots from Aloe explants inoculated in MS basal media supplemented with different concentrations and combinations of BAP and Kn; and IBA at 0.20 mg L\(^{-1}\) were observed within two weeks. Initiation of shoot from explants was started after 4 weeks of inoculation and after inoculating the micro shoots on MS basal medium with different concentration and combination of BAP and Kn (in combination of IBA 0.2 mg/L) proliferation started after 2 weeks of incubation (Bhandari et al., 2010). Similarly, Aloe calli inoculated in MS media supplemented with 1 mg L\(^{-1}\) of BAP and 0.5 mg L\(^{-1}\) of NAA yielded shoots in 2 to 3 weeks and sub culturing was carried out after 20-25 days of inoculation (Choudhary et al., 2011).

Furthermore, Hashemabadi and Kaviani (2008) reported that the best initiation and multiplication of shoots were among explants inoculated on MS medium supplemented with 0.5 mg L\(^{-1}\) BAP + 0.002 mg L\(^{-1}\) NAA and 2 mg L\(^{-1}\) BAP + 0.002 mg L\(^{-1}\) NAA, respectively. Hosseini and Parsa, 2007; Bhandari et al., 2010, reported maximum shooting among explants inoculated in growth media supplemented with 2.0 to 4.0 mg L\(^{-1}\) BAP. In fact, it is well established that BAP is best PGR than other cytokinins that enhance the shoot initiation and proliferation.

Abdi et al. (2013) studied on a method for mass propagation of Aloe vera by using different explants and different media with different PGR. 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\(^{-1}\) NAA and 4 mg l\(^{-1}\) BA in type A explants. Also, the highest shoot proliferation response obtained successfully by using MS medium containing 4 mg l\(^{-1}\) BA. Healthy Aloe vera showing good biomass yield were collected for plant material. Shoots with young leaves were collected from the elite plants.

Haque and Ghosh (2013) studied Nodal portion of rhizomatous stem of A. vera were cultured on Murashige and Skoog (MS) medium. Supplemented with various cytokinin and A. vera leaf gel AvG, 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 6-benzylaminopurine and 10.0% (v/v) AvG, only 17.8 ± 0.35 shoots per explant were induced on 1st regeneration 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. Plants conditions were collected from Nallamalas ranges of the Eastern Ghats Mountains of the Andhra Pradesh state of India and maintained in our experimental garden.

Dwivedi et al. (2014) investigated the rate of explants multiplication was significantly different according to the various concentration of cytokinins supplemented. Treatment with BA 0.5 mg l-1 induced comparable number of shoots. Multiplication rate in the treatment with BAP at 1.0 mg l-1 was intermediate between treatments with 0.5 mg l-1 and 2.5 mg l-1. The best explants multiplication with growth was observed at 1.5 mg l-1 where maximum of 14 explants could be observed per tube. Treatment with 2 mg l-1 gave 7.4 shoots on an average. Average number of 5.4 shoots was obtained on MS media with 2.5 mg l-1. No shoot enhancement was obtained on medium containing Kinetin compared to MS medium. Explant cultures were thus obtained on this selected medium by regular sub culturing at 6 weeks intervals. However, root growth and elongation could be enhanced by supplementing IBA (0.5) mg l-1 with MS medium. Plantlets were hardened after 8 weeks and transferred to potting soil for acclimatization. The explants of A. vera (IC281112, augmented from Jodhpur, Rajasthan) maintained in pots under green house condition at NBPGR Regional Station, Thrissur served as the explant source for the present in vitro plant regeneration experiments.

2.3.5. In vitro root induction

It is well established that the most commonly used PGRs in enhancing rooting of shoots are the auxins. Newly formed, 2 to 3 cm shoots of Aloe inoculated in rooting media supplemented with various concentrations of IAA, IBA, and NAA resulted in rooting, the best being among those inoculated in MS media supplemented with 1.5 mg L\(^{-1}\) IAA. Lee et al., (2011), on the other hand,
reported that NAA at 0.5 mg L\(^{-1}\) was most effective in improving root induction rate, number of adventitious roots per explants, and root length during six weeks of culture. The author reported that supplementation with IBA and IAA showed no or very little adventitious root induction, respectively. Similarly, Saggoo and Kaur (2010) reported that \textit{in vitro} raised shoot tips of \textit{Aloe} cultured on rooting media supplemented with 0.3 mg L\(^{-1}\) of NAA yielded the best rooting response but no rooting was observed in shoot tips cultured on MS medium alone. Root induction response were also found using \textit{Aloe} gel as root induction medium. However, it is well documented that \textit{in vitro} raised shoots can be rooted on PGRs-free MS media (Hosseini and Parsa, 2007; Bhandari \textit{et al.}, 2010).

\textbf{2.3.6. Acclimatization of \textit{in vitro} produced plantlets}

Research reports indicated that acclimatization rates of \textit{Aloes} are quite high. Matured plantlets planted in plastic pots filled with a mixture of coco peat and perlite (1:1) placed in greenhouse (24 ± 1 °C and 70% RH) with periodic irrigation showed excellent rate of acclimatization (Hashemabadi and Kaviani, 2008). Similarly, \textit{in vitro rooted} plantlets planted in a mixture of soil, sand and manure/compost at a ratio of 1:1:1, and maintained under optimum conditions in greenhouse were able to produce hardened plantlets that can be transferred to field. Bhandari \textit{et al.}, (2010) reported that rooted plantlets transferred to polyethylene greenhouse and shade house showed 90% survival and 80% survival rate, respectively, by the end of the first 10 days of acclimatization. Compost, loam, and clay soil at the ratio of 1:2:2 (e.g. Hosseini and Parsa, 2007) and soil and rice husk at a ratio of 1:1 (Saggoo & Kaur, 2010) were also good in enhancing the acclimatization of \textit{Aloe} under greenhouse conditions.
Figure 3.1: Map of Chhattisgarh State

District from where germplasm accessions were collected.
CHAPTER-III
MATERIALS AND METHODS

The present experiment “In vitro regeneration of aloe vera (Aloe barbadensis Mill)”. The research will be conducted in Commercial Tissue Culture Laboratory, IGKV, Raipur, C. G. India. The materials and methods were used during the present investigation are as follows:

3.1 Geographical situation:

Raipur is situated nearly in the central part of Chhattisgarh and lies between 21°16’ N latitude and 81°36’ E longitude with an altitude of 298.60 meters above the mean sea level.

3.2 Experimental Materials:

In Set-I A total 13 accession of aloe vera representing different districts of C.G. The detail of materials used are presented in table 3.1

Table 3.1: Set-I List of germplasm accessions collected from different district of C.G.

| S NO. | District      | No. of Accessions | Zone of C.G. |
|-------|---------------|-------------------|--------------|
| 1     | Bilaspur      | Acc1              | C            |
| 2     | Ambikapur     | Acc2              | N            |
| 3     | Raipur        | Acc3              | C            |
| 4     | Baikunthpur   | Acc4              | N            |
| 5     | Bhilai        | Acc5              | C            |
| 6     | Kondagaon     | Acc6              | B            |
| 7     | Raigarh       | Acc 7             | N            |
| 8     | Champa        | Acc 8             | C            |
| 9     | Dhamtari      | Acc 9             | C            |
| 10    | Jagdalpur     | Acc 10            | B            |
| 11    | Jashpur       | Acc 11            | N            |
| 12    | Raipur        | Acc 12            | C            |
| 13    | Raipur        | Acc 13            | C            |
3.3 Methods

The 13 accession collected from different district of the C.G. During kharif 2016 all the collected accession from different location in herbal garden of IGKV Raipur. The data of transplanting was done on 21.07.2016. The row to row spacing and plant to plant distance maintained at 60 cm in Randomized Block Design (RBD) in three replication. FYM was applied during the field preparation. Irrigation was given as per requirement. Weeding was done as and when required to raise good crop. The present investigation was carried out to assess genetic variability, $h^2$, path, genetic diversity in the aloe vera in the collected accession of C.G. The data obtained from randomly selected following observations were recorded.

**Observations recorded**

The observations were recorded for following traits:

1. **Plant height (cm)**
   The height of the plant was measured in cm from the ground level to tip of primary branches of physiologically matured plant.

2. **Leaf length (cm)**
   The leaf length was measured at the time physiologically matured plant.

3. **Leaf width (cm)**
   The leaf width was measured at the time physiologically matured plant.

4. **Thickness of leaf (cm)**
   The leaf width was measured at the time physiologically matured plant.

5. **No. of spine/plant**
   No. of spine/plant was recorded at the time physiologically matured plant.

6. **No. of leaf/plant**
   No. of leaf/plant was observed at the physiologically matured plant.

7. **No. of suckers per plant**
   No. of suckers per plant was observed at the physiologically matured plant.

8. **Gel content/leaf (g)**
   Gel content/leaf (g) was observed at the physiologically matured plant.

**Qualitative characters**

9. **leaf colour**
Plate 3.1: Experimental field view.
The leaf colour was recorded on mature stage of plant.

1. Light
2. Dark

10 Disease/pest infestation.

The infestation of disease and pest was recorded during the crop growing season.

3.4 Statistical Analysis

3.4.1 Analysis of variance

The data obtained from the individual plant observations from Randomized Block Design experiment were analyzed statistically as per the procedure given by Cochran and Cox (1957).

Table 3.2: The skeleton of variances for Randomized Complete Block Design

| Source     | D. f.          | SS   | Value   |
|------------|----------------|------|---------|
| Replication| (r-1)(t-1)     | SSR  | SR/MSE  |
| Genotype   | r-1)(t-1)      | SST  | ST/MSE  |
| Error      | r-1)(t-1)      | SSE  |         |
| Total      | t-1            | TSS  |         |

Where,

r = Number of replication

\[ \text{r} = \text{number of genotype} \]

To test the significance of treatment, the calculated value of \( F \) was compared with tabular value of \( F \) at 5 and 1 per cent levels of probability against error degree of freedom, \( i.e. (r-1)(t-1) \)

3.4.2 Estimation of genetic parameter of variation

3.4.2.1 Mean

Mean of the character was estimated by summing up of all the observation and dividing the sum by the number of observation.

\[ \bar{X} = \frac{\sum X_i}{n} \]

Where,

\[ \sum X_i = \text{Summation of all the observation} \]
n = Number of observation

3.4.2.2 Range
Rang is the differences between the least and the greatest terms of a series of observation and thus provides the information about the variability present in the genotype.
Range=Highest value-Lowest value

3.4.2.3 Estimation of coefficients of variation
Genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) were calculated by the method suggested by Burton (1952).

3.4.2.3.1 Phenotypic coefficient of variation (PCV)
The estimates of PCV and GCV were classified as low, moderate and high to according Sivasubramanian and Madhavamenon (1973).

\[
\text{GCV}\% = \frac{\sigma^2_g}{X} \times 100
\]

\[
\text{PCV}\% = \frac{\sigma^2_p}{X} \times 100
\]

< 10 per cent = Low

> 20 per cent = High

10 – 20 per cent = Moderate

3.4.2.4 Genetic advance
Improvement in the mean genotypic value of selected plants over the parental population is known as genetic advance. The expected advance was calculated by the formula given by Johnson et al.,(1955) as described below.

\[
\text{GA} = \text{K} . h^2 . \sigma p
\]

Where,

\[
\text{GA} = \text{Genetic advance}
\]

\[
\text{K} = \text{Constant (Standardized selection differential) having value of 2.06 at 5%}
\]
level of selection intensity.
\( h^2 = \) Heritability of the character
\( \sqrt{\sigma_p} = \) Phenotypic Standard deviation

The genetic advance as percentage of mean was estimated as per the below formula:

Genetic advance as percent of mean = \( \frac{\text{Genetic advance}}{\text{General mean}} \times 100 \)

| Range       | Category |
|-------------|----------|
| >20 percent | high     |
| 10-20 percent | moderate |
| <10% percent | low      |

The magnitude of genetic advance as percent of mean was categorized as high (>30%), moderate (30%-10%) and low (<10%)

### 3.4.2.5 Estimation of heritability

Heritability in broad sense (\( h^2_{bs} \)) defined as the proportion of the genotypic variance to the total variance (phenotypic) was calculated as per the formula suggested by Burton (1956)

\[ H^2_{(bs)} \% = \frac{\sigma^2_g}{\sigma^2_p} \]

where,
- \( h^2_{(bs)} = \) Heritability in broad sense
- \( \sigma^2_g = \) Genotypic variance
- \( \sigma^2_p = \) Phenotypic variance

The broad sense heritability estimates were classified as low (<50%), moderate (50-70%) and high (>70%) as suggested by Robinson(1966).

### 3.4.3 Estimation of correlation coefficient

Correlation coefficient analysis measures the mutual relationship between various characters at genotypic (g), phenotypic (p) and environmental levels with the help of formula suggested by Miller *et al.* (1958).

1. Genotypic correlation coefficient character x and y
2. Phenotypic correlation coefficient between character x and y

\[ r_{xy(p)} = \frac{\text{Cov}_{xy(p)}}{\sqrt{\text{Var}_{x(p)} \times \text{Var}_{y(p)}}} \]

Where,

- \( r_{xy(g)} \) = Genotypic correlation coefficient between x and y
- \( r_{xy(p)} \) = Phenotypic correlation coefficient between x and y
- \( \text{Cov}_{xy(g)} \) = Genotypic covariance between x and y
- \( \text{Cov}_{xy(p)} \) = Phenotypic covariance between x and y
- \( \text{Var}_{x(g)} \) = Genotypic variance of x
- \( \text{Var}_{x(p)} \) = Phenotypic variance of x
- \( \text{Var}_{y(p)} \) = Genotypic variance of y
- \( \text{Var}_{y(p)} \) = Phenotypic variances of y

Significance of correlation:
Phenotypic correlation coefficient were tested for their significance following 't' test at n-2 degree of freedom.

\[ t_c = r \sqrt{\frac{n-2}{1-r^2}} \]

If 't' calculated (tc) is \( \geq \) 't' tabulated (tT) at (n-2) degree of freedom at given probability level, the coefficient of correlation is considered as significant.

3.4.4 Path coefficient analysis
Path analysis was originally developed by Wright (1921) and elaborated by Dewey and Lu (1959). Path coefficient analysis splits the genotypic correlation coefficient into measure of direct and indirect effects. It measures the direct and indirect contribution of independent variables on dependent variable. The result of path coefficient analysis is interpreted as per the following scale suggested by Lenka and Mishra (1973).
Table 3.3: Scale for path analysis

| Value of direct and indirect effects | Rate/ Scale |
|-------------------------------------|-------------|
| 0.00 to 0.09                        | Negligible  |
| 0.10 to 0.19                        | Low         |
| 0.20 to 0.29                        | Moderate    |
| 0.30 to 0.99                        | High        |
| > 1.00                              | Very High   |

3.4.5 Genetic divergence analysis

The D² statistic was originally developed by Mahalanobis in 1928. Rao, (1952) suggested the application of this technique for the assessment of genetic divergence in plant breeding. The varieties were grouped into a number of clusters as per the standard procedure described By Spark (1973).

3.5 Experimental and other material

Set II The specimen of Aloe vera will be taken from herbal garden of IGKV, Raipur (C.G). The plant selected was high in gel content in each leaf. The plant population was uniform. In this experiment apical shoot bud, middle part of leaf, leaf spines, leaf tip of Aloe vera will be used for in-vitro regeneration.

3.6 Method

The formulation of murashige & skoog (1962) basal media will be followed through all the experiment as it is most widely used media in contemporary for tissue culture work. The media will be nourished with different hormone concentration and its combination for rooting and shooting. The MS media (HI-media) will be used for making stock solution of 1 litre. The MS media of (HI-media) does not contain sucrose, PVP, agar and hormones. This will be added in MS media.

In The present study of Micropropagation of aloe vera following steps were adopted sequentially:
### 3.6.1 Preparation of stock solutions

The formulation of Murashige and Skoog (1962) basal medium was followed through all the experiments, as it is most widely used media in contemporary for tissue culture work. A general composition of basal MS medium is given in the table.

#### Table 3.4: Chemical Composition of Murashige and Skoog (1962) Media

| Constituents         | Amount (mg/l) present in original medium |
|----------------------|-----------------------------------------|
| **I. Inorganic compound** |                                         |
| (a) Macro nutrients   |                                         |
| KNO$_3$              | 1900                                    |
| NH$_4$NO$_3$         | 1650                                    |
| CaCl$_2$.2H$_2$O     | 440                                     |
| MgSO$_4$.7H$_2$O     | 370                                     |
| KH$_2$PO$_4$         | 170                                     |
| (b) Micro nutrients  |                                         |
| MnSO$_4$.4H$_2$O     | 22.3                                    |
| ZnSO$_4$.7H$_2$O     | 8.6                                     |
| H$_3$BO$_3$          | 6.2                                     |
| KI                   | 0.83                                    |
| Na$_2$MoO$_4$.2H$_2$O| 0.25                                    |
| CoCl$_2$.6H$_2$O     | 0.025                                   |
| CuSO$_4$.5H$_2$O     | 0.025                                   |
| (c) Iron source      |                                         |
| Na$_2$EDTA           | 37.25                                   |
| FeSO$_4$.7H$_2$O     | 27.85                                   |
| **II Organic components** |                                        |
| (a) Vitamins         |                                         |
| Myoinositol          | 100                                     |
| Nicotinic acid       | 0.5                                     |
| Pyridoxin HCL        | 0.5                                     |
| Thiamine HCL         | 0.1                                     |
| (b) Amino acid       |                                         |
| Constituents | Amount to be taken for the stock solution |
|-------------|------------------------------------------|
| (a) Stock C |                                          |
| MgSO\(_4\).7\(\text{H}_2\text{O}\) | 18.5                                     |
| MnSO\(_4\).4\(\text{H}_2\text{O}\) | 1.115                                    |
| ZnSO\(_4\).7\(\text{H}_2\text{O}\) | 0.430                                    |
| CuSO\(_4\).5\(\text{H}_2\text{O}\) | 0.0012                                   |
| (b) Stock D |                                          |
| Ca\(\text{Ch}.2\text{H}_2\text{O}\) | 22.0                                     |
| KI          | 0.0415                                   |
| Co\(\text{Ch}.6\text{H}_2\text{O}\) | 0.0012                                   |
| (c) Stock E |                                          |
| KH\(\text{H}_2\text{PO}_4\)       | 8.50                                     |
| HhBOs       | 0.31                                     |
| Na\(2\text{MoO}_4\). 2 \text{H}_2\text{O}\ | 0.0125                               |
| (d) Stock F |                                          |
| FeSO\(_4\).7\(\text{H}_2\text{O}\) | 1.392                                    |
| Na\(2\) EDTA.2\(\text{H}_2\text{O}\) | 1.8625                                  |
Table 3.6: Stock solutions of hormones

| Hormones | Required amount for stock solution (mg) | Amount of solvent required to dissolve | Amount of water added (ml) |
|----------|----------------------------------------|---------------------------------------|---------------------------|
| NAA      | 10                                     | 1 ml IN NaOH                           | 100ml                     |
| IBA      | 10                                     | 1 ml IN NaOH                           | 100ml                     |
| BAP      | 10                                     | 1 ml IN NaOH                           | 100ml                     |
| IAA      | 10                                     | 1 ml IN NaOH                           | 100ml                     |

3.6.2 Stock solution of vitamins

To make 50 ml of vitamins stock solution, 25 mg of nicotinic acid was dissolved in 25 ml of boiling distilled water and the final volumes was made to 50 ml and allow to cool and stored in refrigerator at 0 ºC for 10 days. Similarly the other two vitamins pyridoxine HCl (25mg) and thiamine HCl (5mg) were prepared and stored.

3.5.3 Stock solutions of amino acid

Hundred milligram of glycine was dissolved in 50 ml double distilled water and stored at 0 ºC for maximum period of 15 days.

3.6.4 Stock solution of hormones

Hormones were not directly dissolved in water so they were at first made soluble in water miscible solvents and then water was added to get final volume. Fifty mg of auxin Indole 3-butyric acid (IBA) and Indole acetic acid (IAA) were initially dissolved in the 1 ml of absolute ethanol and then volume was made up to the final volume of 50 ml by adding double distilled H2O. Fifty mg of cytokinin 6-benzyl amino purine (BAP) were first dissolved in 1 ml 1N NaOH and final volume was made up to 50 ml with double distilled water.
3.6.5 Preparation of culture media.

1 litre water (Magnetic stirrer 20 minute)

→ MS media 4.41 gm will be taken

→ Sucrose 30 gm

→ Hormones will be added

   BAP

   NAA -2 ml

→ 0.50 gm PVP will be added

→ Volume make up to 1 liter

→ pH will be adjusted to 7.5 to 8.0

   1N NaOH – High

   HCl – Low

→ Heat in gas

→ Add 7.5 gm agar for solution to make semisolid

→ Pouring in culturing bottle up to 1.5 inch

→ Sterilized in and autoclave
Plate 3.2: Laboratory works view
3.6.6 Preparation of explants.

Young, healthy of 1-2 month old plant of aloe vera was taken from herbal garden. These plants were then washed thoroughly with tap water and dipped 7% labolene liquid detergent and 1% bavistin solution for 5 minutes to remove the attached mud and soil. All traces of labolene and bavistin were removed by repeated washings in running tap water. The older leaves and roots were carefully removed with a stainless steel knife. apical shoot bud, middle part of leaf, leaf spines, leaf tip of Aloe vera will be used for in-vitro regeneration measuring about 1.5 cm in length were carefully excised. These excised explants were surface sterilized and then inoculated in the MS media in aseptic condition i.e. in laminar flow hood chamber.

3.6.7 Surface sterilization of explants.

All the explants from plant of aloe vera was thoroughly washed in labolene and bavistin solution to clear the dirt particles.
The explants were treated with mercuric chloride and then with ethyl alcohol for certain time period Then the explants were washed with double distilled water at least 3-4 times after each chemical treatment and inoculation in MS media. All the operations during inoculation including surface sterilization of meristematic shoot tip were carried out inside the laminar air flow cabinet.

3.6.8 Inoculation of explants.

After sterilization, the explants were kept on sterilized bottle. The explants were cut horizontally from the top using a sterile blade. After it explants were transferred in MS culture initiation media containing cytokinin 6-Benzyl aminopurine (BAP) + NAA hormone of varied concentration After 3 weeks shoots were formed. They were separated and sub cultured in media containing different concentration of half BAP+NAA with the best six shoot initiation media selected on the basis of performance of shoot initiation response and mean number of shoot initiated.
### Table 3.7: Treatments for surface sterilization of explant

| Treatment no. | Sterilants      | Percent of Sterilants | Time Required |
|---------------|-----------------|-----------------------|---------------|
| 1             | Labolene        | 7.0%                  | 5 minute      |
| 2             | Bavistin        | 1%                    | 5 minute      |
| 3             | Ethanol         | 70%                   | 30 seconds    |
| 4             | HgCl<sub>2</sub>| 0.1%                  | 20 minute     |
| 5             | Sodium hypochloride | 1.5%              | 20 minute     |

### Table 3.8: Different treatments for shoot initiation

| S. No. | Treatment Code | Hormones Concentration |
|--------|----------------|------------------------|
| 1      | T<sub>1</sub>  | MS media+ 3BAP+0.2NAA  |
| 2      | T<sub>2</sub>  | MS media+ 5BAP+0.2NAA  |
| 3      | T<sub>3</sub>  | MS media+ 8BAP+0.2NAA  |
| 4      | T<sub>4</sub>  | MS media+ 2BAP+0.2NAA  |
| 5      | T<sub>5</sub>  | MS media+ 4BAP+0.2NAA  |

### 3.6.9 Incubation and maintenance of culture

All the aloe vera explants cultures containing the newly emerged shoots in bottles were incubated in racks inside a culture room with controlled condition of light, temperature and humidity. The culture were exposed alternatively into photoperiodic cycle of 14 hours cool white fluorescent light and 10 hours dark at 25 ± 2°C and 50 % relative humidity maintained inside the culture room.

### 3.6.10 Rooting

After five to six sub-culturing, the shoots were separated and transferred to the different concentrations and combinations of rooting
Table 3.9: Different treatments for rooting

| S.N. | Treatment code | Hormone concentration                                      |
|------|----------------|------------------------------------------------------------|
| 1    | T₁             | Basal MS( half ) medium +500 mg/l activated charcoal      |
| 2    | T₂             | MS( half ) + 0.5 mg/l IBA + 500mg/l activated charcoal    |
| 3    | T₃             | MS( half ) + 0.5 mg/l IAA + 500mg/l activated charcoal    |
| 4    | T₄             | MS( half ) + 0.5 mg/l IBA                                  |

3.6.11 Acclimatization of Plants and Transfer of Regenerants to Field

After the root formation, the plants started elongation. After 15 days the plants were ready to transfer in polyhouse. After rooting, plantlets with sufficient roots were removed from the culture medium carefully, washed thoroughly till complete media was removed from the plantlets surface. The rooted plantlets were transferred to the nursery plate containing vermiculate supplemented with half strength MS liquid medium. The seedlings were covered with perforated polythene bags and maintained for 30 days, after acclimatized seedlings in the plastic try were transferred to net house with vermiculate, FYM and sand (1:1:1) in the pots in order to maintain minimum 60-65 per cent humidity for second hardening.
Figure 3.2: Meteorological data

- max temperature (°C)
- min temperature (°C)
- rainfall
- relative humidity max
- Relative humidity max
- wind velocity
- evaporation
- sunshine
CHAPTER IV
RESULTS AND DISCUSSION

The present investigation was carried out to assess genetic variability, path, genetic diversity in the aloe vera collected from different parts of C.G. The aloe vera plant propagated through sucker, these are limited in numbers and varies in age due to their for uniform harvesting of the produce is affected. Therefore suitability of in vitro technique for the micropropagation of aloe vera using different explants viz leaf tip, leaf spines and apical shoot bud of 2-3 month old field grown *Aloe barbadensis* plants were exposed to various concentration of hormones NAA, BAP for the establishment of high efficiency protocol for the production of *Aloe barbadensis* plantlets *in vitro*.

The results obtained in the present investigation On “In vitro regeneration of aloe vera (Aloe barbadensis)” are discussed here under following heads.

4.1: Mean performance of genotype and analysis of variance.

4.2: Estimation of genetic variability.

4.3: Genetic divergence analysis.

4.4: Association analysis.

4.4.1: Correlation coefficient analysis.

4.4.2: Path analysis

4.5: Standardization of protocol for high frequency in vitro regeneration of aloe vera. *In - vitro* culture of plants using different explants.

4.5.1: Surface sterilization technique.

4.5.2: Combination of plant growth regulator on shoot initiation.

4.5.3: Combination of plant growth regulator on root initiation.

4.5.4: Acclimatization of plants and transfer of regenerates to field.
4.1: Mean performance of genotype and analysis of variance:

The Average performances of the 13 aloe barbadensis genotype are shown in the table 4.1. Analysis of variance was worked out for high gel content and its attributing traits indicated that the mean sums of squares due to genotypes were highly significant for plant height (cm), leaf length (cm), leaf width (cm), thickness of leaf (cm), no. of spine/plant, no. of leaf/plant, no. of suckers per plant and gel content per leaf(g). Significant mean sum of squares due to gel content (g) per leaf and attributing character revealed that existence of considerable variability in the material studied for the improvement of various traits and better chances of improvement through selection on the basis of their traits.

Table 4.1: Analysis of variance for different character of aloe vera

| S. No. | Sources of Variation     | Degree of freedom | Replication 2 Mean Sum of Squares | Genotype 12 Mean Sum of Squares | Error 24 Mean Sum of Squares |
|--------|--------------------------|-------------------|-----------------------------------|--------------------------------|-----------------------------|
| 1.     | Plant height (cm)        |                   | 92.026**                         | 408.966**                      | 25.248                      |
| 2.     | Leaf length (cm)         |                   | 14.795**                         | 225.979**                      | 23.850                      |
| 3.     | Leaf width (cm)          |                   | 0.182                            | 2.374                          | 0.421                       |
| 4.     | Thickness of leaf (cm)   |                   | 0.005                            | 0.055                          | 0.011                       |
| 5.     | No. of spine/plant       |                   | 26.564**                         | 313.248**                      | 10.453                      |
| 6.     | No. of leaf/plant        |                   | 3.308                            | 21.299**                       | 3.197                       |
| 7.     | No. of suckers per plant |                   | 0.718                            | 143.521**                      | 2.162                       |
| 8.     | Gel content in per leaf(g)|                 | 1,474.956**                     | 4,833.364**                    | 408.295                     |

4.2: Estimation of genetic variability:

Genetic parameters of variation are presented in Table 4.2 for all the characters. The overall mean and range for gel content and its components revealed that there are suitable genetic variability present for most of the characters among the germplasm accession under study. Genetic parameters of variation are discussed character wise.

4.2.1 Character mean and range
4.2.1.1 Plant height (cm)

The plant height (cm) ranged from 20 to 65 cm with an average plant height 46.43 cm. Among all Acc.-12(65cm.) was recorded as maximum and Acc-11(20cm) was recorded as the minimum plant height.
4.2.1.2 Leaf length (cm)

The leaf length ranged was recorded 16 to 53 cm, with an average Leaf length (cm) 34.48 cm. Among all Acc.-12 was recorded longest in leaf length with (53cm.) and Acc-11 was recorded the short leaf length with 16 cm.

4.2.1.3 Leaf width (cm)

The leaf width ranged was recorded 4.0 to 7.9 cm. with an average Leaf width with 5.82 cm. Among all acc.-12 was recorded longest in leaf width with (7.9 cm.) and acc-11 was recorded the short leaf length with 4.0 cm.

4.2.1.4 Thickness of leaf (cm)

The Thickness of leaf (cm) ranged was recorded 0.2 to 0.8 cm, with an average Thickness of leaf (cm) 0.54 cm. Among all acc.-12 was recorded longest in leaf length with (0.8 cm.) and acc-11 was recorded the short leaf length with 0.2 cm.

4.2.1.5 No. of spine/plant

The No. of spine/plant ranged was recorded 12 to 56. with an average No. of spine/plant 29.64. Among all acc.-12 was recorded maximum (56) No. of spine/plant and acc-11 was recorded the minimum with 12 No. of spine/plant.

4.2.1.6 No. of leaf/plant

The No. of leaf/plant ranged was recorded 6 to 18, with an average No. of leaf/plant 10.76. Among all acc.-13 was recorded maximum in No. of leaf/plant (18) and acc-11 was recorded the minimum No. of leaf/plant with 6.

4.2.1.7 No. of suckers per plant

The No. of suckers per plant ranged was recorded 02 to 32 with an average No. of suckers per plant 6.32. Among all acc.-12 was recorded maximum in No. of suckers per plant and Acc-11 was recorded the minimum No. of suckers per plant with.

4.2.1.8 Gel content/leaf (g)

The Gel content (g) ranged was recorded 15.1 to 178.4 (g) with an average Gel content (g) 77.17 g. Among all acc.-1 was recorded maximum in gel
content/leaf (g). and Acc-11 was recorded the minimum gel content per leaf (g) with 21.8 cm.

4.2.2 Genotypic and phenotypic coefficient of variation

Genotypic and phenotypic coefficient of variation is simple measures of variability these measures are commonly used for the assessment of variability. The related value of this type of coefficient gives an idea of magnitude of variability present in a population. Thus the component of variation such as Genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) were computed. Categorized as low (less than 10%), moderate (10-20%) and high (more then 20%) as suggested by Sivasubramanian and Madhavamenon (1973).

In present Investigation the PCV value was slightly higher than GCV which showed that the trait was influenced by the environment. Among the different gel content per plant and its attributing traits, number of suckers per plant had highest magnitude of GCV (96.65) and PCV (98.07) followed by number of spines per plant (GCV 33.894) and (PCV 35.606) followed by plant height (cm) (GCV 24.355) and (PCV 26.651), followed by Leaf length (cm) (GCV 23.80) and (PCV 27.695), followed by Thickness of leaf (cm) (GCV 22.337) and (PCV 29.507) followed by No. of leaf/plant (GCV 22.810) and (PCV 28.212), followed by Gel content per leaf (g) (GCV 49.767) and (PCV 56.235). The high moderate GCV and PCV were observed for leaf width (13.842 and 17.766%).

4.2.3 Heritability and Genetic advance as percentage of mean

Heritability estimates provide the information regarding the amount of transmissible genetic variation to total variation and determine genetic improvement and response to selection. Thus, heritability is the heritable portion of the phenotypic variance. It is a good index of the transformation of characters from parent to their off springs. Heritability and genetic advance are important selection parameters. Improvement in the mean genotypic value of selected plants over the parental population is known as genetic advance. It is the measure of genetic gain under selection. The success of genetic advance under selection depends on genetic variability, heritability and selection intensity.
In present investigation high magnitude of heritability was recorded for most of the traits. The highest variability was recorded for the traits e.g. plant height (cm) (83.515), leaf length (cm) (73.856), no. of spine/plant (90.615), no. of suckers/plant (95.112) and gel content/plant (g) (78.320). It indicated that these character is least influenced by the environment or therefore selection of such character will be rewarded.

Genetic advance is a measure of genetic gain under selection. The success of genetic advance under selection depends on heritability of the character under consideration. This indicates that though the character is less influenced by environmental effects, the selection for improvement of such trait may not be useful because, heritability is based on total genetic variance which includes fixable (additive) and non fixable (dominance and epistatic) variance. The magnitude of genetic advance as percent of mean was recorded high for all the traits. Only some traits observed moderate genetic advance plant height (21.291%), Leaf length (cm) (14.532%), Leaf width (cm) (1.295%), Thickness of leaf (cm) (0.189%), No. of spine/plant (19.701%), No. of leaf/plant (4.091%), suckers (13.827%), gel content (70.017%).

All the traits possessing high values of genetic advance indicate that the characters are governed by additive genes and selection will be rewarding for improvement of such trait.

High heritability coupled with high genetic advance as percentage of mean was found in the character gel content/plant (78.32 and 70.01). It indicates that heritability is due to additive gene effect or selection of such character may be effective. High heritability coupled with moderate genetic advance as percentage of mean was found in trait plant height (cm) (83.515 and 21.291) leaf length (73.856) and (14.532), No. of spine/plant (90.615) and (19.70), No. of suckers per plant (95.612) and (13.827). It showed that the character is govern by additive genes and selection will be rewarding for improvement of such trait.
Table 4.2: Estimation of genetic parameter for different trait in aloe vera.

| S.NO. | character                             | Mean  | Range | Standard Error | GCV (%) | PCV (%) | Heritability | Genetic Advance |
|-------|---------------------------------------|-------|-------|----------------|---------|---------|--------------|-----------------|
|       |                                       |       | Min.  | Max.           |         |         |              |                 |
| I     | Plant height (cm)                     | 46.43 | 20    | 65            | 4.103   | 24.355  | 26.651       | 83.515          |
| II    | Leaf length(cm)                       | 34.48 | 16    | 53            | 3.988   | 23.801  | 27.695       | 73.856          |
| III   | Leaf width (cm)                       | 5.82  | 4.00  | 7.9           | 0.530   | 13.842  | 17.766       | 60.709          |
| IV    | Thickness of leaf(cm)                 | 0.54  | 0.2   | 0.8           | 0.086   | 22.337  | 29.507       | 57.309          |
| V     | No. of spine per leaf                 | 29.64 | 12    | 56            | 2.640   | 33.894  | 35.606       | 90.615          |
| VI    | No. of leaf per plant                 | 10.76 | 06    | 18            | 1.460   | 22.810  | 28.212       | 65.370          |
| VII   | No. of suckers per plant              | 6.43  | 02    | 32            | 1.201   | 96.65   | 98.07        | 95.612          |
| VIII  | Gel content per leaf(g)               | 77.17 | 15.1  | 178.4         | 16.498  | 49.767  | 56.235       | 78.320          |
4.3 Genetic divergence analysis

The choice of genetically diverse parents for hybridization is an important feature of any crop improvement programme for getting desirable segregant. The multivariate analysis based on Mahalanobis $D^2$ or non-Hierarchical Euclidean cluster analysis is used for divergence analysis. The $D^2$ analysis classifies the genotype into relatively homogeneous groups in such a way that within cluster diversity is minimized and between clusters diversity is maximized. The respective genotypes from diverse cluster can be utilized in breeding programme depending upon breeding objectives. The results of earlier studied are relevant only for the material and environmental involved in a particular study and cannot be generalized. Therefore, study on genetic divergence on the available germplasm under the environment where it is to be exploited is essential for successful utilization of available resources. Genetic diversity is an important factor and also a prerequisite in any hybridization programme. Multivariate analysis by mean of Mahalanobis $D^2$ statistic is a powerful tool in quantifying the degree of divergence at genotypic level. Vavilov (1926) was the first to emphasize the need for a broad genetic base for crop improvement. A set of 13 genotypes of aloe vera were subjected to $D^2$ analysis for 8 characters based on $D^2$ values four cluster were formed. This indicated that substantial diversity existed in the all the genotypes evaluated in the present study. This present study also suggests that, there is no relationship between geographical and genetic diversity as genotype chosen from different eco-geographical regions are grouped in different clusters. Cluster IV were the largest which consisted of 5 accession, followed by cluster II, with 4 accession followed by cluster I with 3 accession and cluster III (1 genotype). From the clustering pattern, it was found that the genotype from different region were independent of their genetic region. Hence the genotypes studied are reliable enough for hybridization and selection.

The inter and intra cluster distance among the four cluster are presented in table 4.4 and fig 4.1. The maximum inter cluster distance was observed in between I and IV (5.350) followed by cluster IV and III (3.569). This suggested that the hybridization programme involving parents from these cluster is expected to give higher frequency of better segregates or desirable combination for development of
Table 4.3: Genotype of aloe vera in different cluster

| Cluster  | No. of genotypes | Name of genotype                  |
|----------|------------------|-----------------------------------|
| Cluster I| 3                | Acc 7, Acc 10, Acc 11             |
| Cluster II| 4              | Acc 5, Acc 6, Acc 8, Acc 9        |
| Cluster III | 1          | Acc 4                            |
| Cluster IV | 5           | Acc 1, Acc 2, Acc 3, Acc 12, Acc 13 |

Table 4.4: Average intra-cluster among 4 cluster of aloe vera

| Cluster | I     | II    | III   | IV    |
|---------|-------|-------|-------|-------|
| I       | 1.298 |       |       |       |
| II      | 2.883 | 1.005 |       |       |
| III     | 2.940 | 2.667 | 1.03  |       |
| IV      | 5.350 | 2.941 | 3.567 | 2.183 |
Figure 4.1: Average intra-cluster among 4 cluster of aloe vera.
Table 4.5: Mean performance of genotype in individual cluster for different trait.

| Cluster | Entries | Plant height (cm) | Leaf length (cm) | Leaf width (cm) | Thick of leaf (cm) | No. of spine/plant | No. of leaf/plant | No. of suckers | Gel content in (g) per plant |
|---------|---------|-------------------|------------------|-----------------|-------------------|-------------------|------------------|---------------|-----------------------------|
| I       | 3       | 32.00             | 22.89            | 4.89            | 0.39              | 20.00             | 7.67             | 3.33          | 33.18                       |
| II      | 4       | 47.42             | 33.25            | 6.17            | 0.49              | 25.50             | 11.33            | 4.00          | 55.67                       |
| III     | 1       | 34.00             | 30.00            | 5.33            | 0.63              | 26.00             | 8.00             | 5.00          | 113.77                      |
| IV      | 5       | 56.80             | 43.33            | 6.22            | 0.66              | 39.47             | 12.73            | 10.53         | 113.45                      |

Table 4.6: Desirable genotype based on cluster performance

| Characters                  | I                  | II                 | III                | IV                 |
|-----------------------------|--------------------|--------------------|--------------------|--------------------|
| Plant height (cm)           | Acc10,             | Acc5, Acc8, Acc9   | Acc4               | Acc1, Acc2         |
| Leaf length (cm)            | Acc7, Acc10        | Acc5, Acc8, Acc9   | Acc4               | Acc1, Acc2, Acc3, Acc12, Acc13 |
| Leaf width (cm)             | Acc7, Acc10        | Acc5, Acc8, Acc9   | Acc4, Acc1, Acc3   |                    |
| Thickness of leaf (cm)      | Acc11              | Acc5               | Acc4               | Acc2, Acc13        |
| No. of spine/plant          | Acc7               | Acc8               | Acc4               | Acc2, Acc3, Acc12  |
| No. of leaf/plant           | Acc7, Acc10        | Acc5, Acc8         | Acc4               | Acc1, Acc3         |
| No. of suckers              | -                  | Acc5               | -                 | Acc12, Acc13       |
| Gel content (g)/per plant   | Acc7               | Acc6               | Acc4               | Acc1, Acc3         |
useful genetic stocks or varieties. The minimum inter cluster distance was observed in between II and III (2.667) followed by cluster I and II (2.883). The maximum intra cluster distance was observed in cluster IV (2.183) followed by cluster I (1.298), cluster II (1.5), cluster III (1.03)

4.4: Association analysis

4.4.1: Correlation coefficient analysis

Association analysis is an important approach in breeding program. It gives an idea about relationship among the various characters and determines the component characters, on which selection can be based for genetic improvement in herbage yield. Degree of association also affects the effectiveness of selection process. The degree of association between independent and dependent variables was suggested by Galton 1888, its theory was developed by Pearson (1904) and their mathematical utilization at phenotypic and genotypic and environmental levels was described by Searle (1961).

The Correlation coefficients analysis is the index of association between two variables. These have been dealt in all possible combination for important character at phenotypic, genotypic and environmental level are presented in table no. 4.7

Correlation analysis clearly revealed that the phenotypic correlation and genotypic correlation is general and similar in direction but the magnitude of genotypic correlation was higher than the phenotypic correlation means that there is strong association between these two character genetically. Their primary utility is in strengthening based on genotypic correlation and in better predicating correlated response to selection. Hence important finding based on phenotypic correlation are described below:

4.4.1.1 Correlation of attributing character with gel content in leaf:

4.4.1.1.1 Plant height (cm)

Plant height had found positive and significant Correlation at genotypic and phenotypic level for leaf length (0.930, 0.781), leaf width (0.764, 0.559), thickness of leaf (0.882, 0.617), number of spines (0.821, 0.765), number of
Table 4.7: Genotypic and phenotypic correlation coefficient

| Character                  | Plant height(cm) | leaf length | Leaf width | Thickness of leaf | No. of spine/plant | No. of leaf/plant | No. of suckers | Gel content / plant(g) |
|----------------------------|------------------|------------|------------|-------------------|--------------------|------------------|---------------|------------------------|
| Plant height(cm)           |                  |            |            |                   |                    |                  |               |                        |
| G                         | 1.000            | 0.930**    | 0.764**    | 0.882**           | 0.821**            | 0.948**          | 0.428**       | 0.421**                |
| P                         | 1.000            | 0.781**    | 0.559**    | 0.617**           | 0.765**            | 0.802**          | 0.417**       | 0.579**                |
| E                         | 1.000            | 0.243      | 0.057      | 0.025             | 0.402**            | 0.426**          | 0.474**       | 0.351*                 |
| leaf length                |                  |            |            |                   |                    |                  |               |                        |
| G                         | 1.000            | 0.720**    | 0.973**    | 0.793**           | 0.830**            | 0.415**          | 0.889**       |                        |
| P                         | 1.000            | 0.558**    | 0.535**    | 0.743**           | 0.641**            | 0.395*           | 0.628**       |                        |
| E                         | 1.000            | 0.237      | -0.295     | 0.604**           | 0.213              | 0.429**          | 0.202         |                        |
| Leaf width                 |                  |            |            |                   |                    |                  |               |                        |
| G                         | 1.000            | 0.682**    | 0.320*     | 0.646**           | -0.295             | 0.695**          |               |                        |
| P                         | 1.000            | 0.293      | 0.279      | 0.414**           | -0.194             | 0.442**          |               |                        |
| E                         | 1.000            | -0.267     | 0.218      | 0.020             | 0.233              | -0.127           |               |                        |
| Thickness of leaf          |                  |            |            |                   |                    |                  |               |                        |
| G                         | 1.000            | 0.823**    | 0.662**    | 0.390*            | 0.908**            | 0.692**          |               |                        |
| P                         | 1.000            | 0.524**    | 0.557**    | 0.283             | 0.442*             | 0.517**          |               |                        |
| E                         | 1.000            | -0.347*    | 0.395*     | -0.040            | 0.277              |                  |               |                        |
| No. of spine/plant         |                  |            |            |                   |                    |                  |               |                        |
| G                         | 1.000            | 0.930**    | 0.816**    | 0.567**           |                    |                  |               |                        |
| P                         | 1.000            | 0.752**    | 0.768**    | 0.501**           |                    |                  |               |                        |
| E                         | 1.000            | 0.201      | 0.134      | 0.165             |                    |                  |               |                        |
| No. of leaf/plant          |                  |            |            |                   |                    |                  |               |                        |
| G                         | 1.000            | 0.613**    | 0.467**    |                  |                    |                  |               |                        |
| P                         | 1.000            | 0.553**    | 0.437**    |                  |                    |                  |               |                        |
| E                         | 1.000            | 0.555**    | 0.376*     |                  |                    |                  |               |                        |
| No. of suckers             |                  |            |            |                   |                    |                  |               |                        |
| G                         | 1.000            | 1.000      | 1.000      |                  |                    |                  |               |                        |
| P                         | 1.000            | 1.000      | 1.000      |                  |                    |                  |               |                        |
| E                         | 1.000            | 1.000      | 0.169      |                  |                    |                  |               |                        |
| Gel content / plant(g)     |                  |            |            |                   |                    |                  |               |                        |
| G                         |                   |            |            |                   |                    |                  |               | 1.000                  |
| P                         |                   |            |            |                   |                    |                  |               | 1.000                  |
| E                         |                   |            |            |                   |                    |                  |               | 1.000                  |

(* = at 5% level of significance) and (**) = at 1% level of significance)
4.4.1.1.2 Leaf length (cm)

Leaf length (cm) had found positive and significant Correlation at both level of genotypic and phenotypic level of plant height (0.830, 0.641), leaf width (0.823, 0.524), number of spine (0.823, 0.524), number of leaf per plant (0.662, 0.557), number of suckers (0.390) was found genotypic significant, gel content per leaf (g) (0.908, 0.692) respectively.

4.4.1.1.3 Leaf width (cm)

Leaf width (cm) had found positive and significant Correlation at both level of genotypic and phenotypic level plant height (cm) (0.764, 0.559), leaf length (cm) (0.720, 0.558), thickness of leaf, number of spine had only genotypic significant (0.682,0.320) respectively, number of leaf per plant (0.646, 0.414), number of suckers (0.415, 0.395), gel content per leaf (g) (0.695, 0.442 respectively).

4.4.1.1.4 Thickness of leaf (cm)

Thickness of leaf had found positive and significant Correlation at both level of genotypic and phenotypic level for plant height (cm) (0.882, 0.617), leaf length (0.973, 0.535), leaf width had only genotypic significant (0.682), number of spine (0.823, 0.524), number of leaf per plant (0.662, 0.557), number of suckers (0.390) was found genotypic significant, gel content per leaf (g) (0.908, 0.692) respectively.

4.4.1.1.5 Number of spine/leaf

Number of spine had found positive and significant Correlation at both level of genotypic and phenotypic level Plant height (cm) (0.821, 0.765), leaf length (cm) (0.793,0.743), leaf width(cm) had only genotypic significant (0.320), Thickness of leaf(0.823, 0.524), number of leaf per plant (0.930, 0.752), number of suckers(0.816, 0.768), gel content per leaf (g) (0.567, 0.501 respectively).

4.4.1.1.6 Number of leaf /plant

Number of leaf per plant had found positive and significant Correlation at both level of genotypic and phenotypic level Plant height (cm) (0.948, 0.802), leaf...
4.4.1.1.7 Number of suckers/plant

Number of suckers had found positive and significant Correlation at both level of genotypic and phenotypic level Plant height (0.421, 0.417), leaf length (0.415, 0.395), leaf width (0.415, 0.395), Thickness of leaf (0.390) had only genotypic significant Number of spine (0.816, 0.768), Number of leaf per plant (0.613, 0.553).

4.4.1.1.8 Gel content/leaf (g)

Gel content had found positive and significant Correlation at both level of genotypic and phenotypic level Plant height (cm) (0.634, 0.579), leaf length(cm) (0.889, 0.628), leaf width (cm) (0.695, 0.442), Thickness of leaf (0.908, 0.692), Number of spine (0.567, 0.501), Number of leaf per plant (0.567, 0.501)

Hence direct selection for plant height (cm.), leaf length (cm.), leaf width (cm.), thickness of leaf, number of spines/leaf, number of leaf/plant may be advantageous for selection of high yield genotype in aloe vera from the available gene pool.

4.4.2: Path coefficient analysis

Path coefficient analysis is simply a standardized partial regression coefficient, which splits the correlation into direct and indirect effect. In other words, it measures the direct and indirect contribution of various independent characters on dependent characters on a dependent character. Following method given by Dewey and Lu (1959) has been used to estimate the magnitude and direction of direct and indirect effect of gel content per plant contributing characters. Correlation coefficients along with path coefficients together provide more reliable information, which can be effectively predicted in crop improvement programme. If the correlation between yield and a character is due to direct effect of a character, it reveals true relationship between them and direct selection for the trait will be rewarding for yield improvement. In gel content per plant however, if the correlation coefficient is mainly due to indirect effect of the character through another component trait, indirect
selection through such trait will be effective in yield improvement. If the direct effect is positive and high, but such trait should be practiced to reduce the undesirable indirect effect.

The path analysis in present investigation revealed that leaf length (cm), leaf width (cm), thickness of leaf (cm), no. of spines, no. of leaf/plant, indicate the true effect and is not affected by any other component of characters and environment. Thus we can conclude that the association was true of such character and direct effect of these characters on gel content in leaf (g) was the major causal in determining the various correlation coefficient estimated and the rate of indirect effect. Therefore direct selection for this character will be beneficial in improving the gel content in leaf (g) in aloe vera.

Path coefficient analysis considering high gel content per leaf (g) as dependent character revealed that No. Of spine per plant (0.659) showed highest positive direct effect followed by no. of leaf per plant (0.445) followed by leaf width (0.367) and leaf length (cm) (1.922).

The other important show only negative direct effect on high gel content per leaf (g). Plant height (-2.493) showed highest negative effect followed by no. of suckers.

4.4.2.1 Plant height (cm)

The character plant height had negligible negative direct effect (-2.493) on gel content per leaf (g) and had positive indirect effect via leaf length (cm) (1.787) and high negative indirect effect via number of suckers (-0.188). it exhibited positive and significant correlation with gel content per leaf (g)

4.4.2.2 Leaf length (cm)

The trait leaf length (cm) exhibited significant positive correlation with gel content per leaf (g). The character leaf length (cm) had positive direct effect (1.922) on gel content per leaf (g) and had negative indirect effect via plant height (cm) (-2.318) and high negative indirect effect via number of suckers (-0.185).
|                          | Plant height (cm) | leaf length | Leaf width | Thickness of leaf | No. of spine/plant | No. of leaf/plant | No. of suckers | Genotypic correlation |
|--------------------------|-------------------|-------------|------------|-------------------|--------------------|------------------|---------------|-----------------------|
| Plant height             | -2.493            | 1.787       | 0.281      | 0.285             | 0.541              | 0.422            | -0.188        | 0.421**               |
| leaf length              | -2.318            | 1.922       | 0.264      | 0.314             | 0.522              | 0.369            | -0.185        | 0.889**               |
| Leaf width               | -1.906            | 1.384       | **0.367**  | 0.220             | 0.211              | 0.287            | 0.132         | 0.695**               |
| Thickness of leaf        | -2.199            | 1.871       | 0.251      | **0.323**         | 0.542              | 0.294            | -0.174        | 0.908**               |
| No. of spine/plant       | -2.048            | 1.524       | 0.118      | 0.266             | **0.659**          | 0.414            | -0.364        | 0.567**               |
| No. of leaf/plant        | -2.363            | 1.595       | 0.237      | 0.214             | **0.445**          | -0.274           | 0.467**       |                       |
| No. of suckers           | -1.050            | 0.798       | -0.108     | 0.126             | 0.538              | 0.273            | **-0.447**    | NS                    |

**RESIDUAL EFFECT** = 0.1997  Figures in bold are direct effects
4.4.2.3 Leaf width (cm)

The trait leaf width (cm) exhibited significant positive correlation with gel content per leaf (g). The character leaf length had positive direct effect (0.367) on gel content per leaf (g) and had negative indirect effect via plant height (cm) (-1.906).

4.4.2.4 Thickness of leaf (cm)

The trait Thickness of leaf exhibited significant positive correlation with gel content per leaf (gm). The character Thickness of leaf had positive direct effect (0.323) on gel content and had negative indirect effect via plant height (cm) (-2.199) and high negative indirect effect via number of suckers per plant(-0.174).

4.4.2.5 No. of spine per plant

The trait No. of spine exhibited significant positive correlation with gel content per leaf (g). The character No. of spine positive direct effect (0.659) on gel content and had negative indirect effect via plant height (cm) (-2.048) and high negative indirect effect via number of suckers (-0.364).

4.4.2.6 Number of leaf per plant

The trait No. of leaf per plant exhibited significant positive correlation with gel content per leaf (g). The character No. of spine positive direct effect (0.445) on gel content and had negative indirect effect via plant height (cm) (-2.363) and high negative indirect effect via number of suckers (-0.274).

4.4.2.7 Number of suckers per plant

The character Number of suckers had negligible negative direct effect (-0.447) on gel content and had positive indirect effect via leaf length (0.798) and high negative indirect effect via leaf width (-0.108). It exhibited non significant correlation with gel content per leaf (g).

4.5: Standardization of protocol for high frequency in vitro regeneration of aloe vera. In - vitro culture of plants using different explants

4.5.1 Effect of surface sterilization treatments

Establishment of contamination free cultures is the first step in development of the regeneration protocol for any plant species. Four explants i. e
apical shoot bud, meristem root tip, leaf tip and spine were excised from aloe vera plants. Initially all the excised plant tissues were washed thoroughly with 7 per cent Labolene and rinsed thoroughly with sterile double distilled water. All the explants were sterilized by using three different surface sterilization treatments. Combination to get contamination free culture of *Aloe barbadensis*. The treatment combination 1% Bavistin for 30 min. The explants was dipped followed by 1% HgCl$_2$ for 45 min followed by 2% concentration of NaOCl for 20 min followed by 70% ethanol for 30sec as found to be best response in getting the contamination free culture.

Earlier Dwivedi *et al.* (2014) have also reported the effect of time and concentration of sterilant to get contamination free culture.

**Table 4.9: Surface sterilization treatments for different explants.**

| Treatment No. | Name of the sterilant | Surface sterilization treatments | Response |
|---------------|-----------------------|----------------------------------|----------|
| I             | Bavistin              | 1%                               | More than 50% cultures responded |
|               | HgCl$_2$              | 1%                               | 5 min.  |
|               | NaOCl                 | 2%                               | 5 min.  |
|                | Bavistin              | 1%                               | 30 min. |
| II            | HgCl$_2$              | 1%                               | 45 min. |
|               | NaOCl                 | 2%                               | 20 min. |
|               | Ethanol               | 70%                              | 30 sec. |

**4.5.2 Effect of different type of explants on shoot initiation**

In a pilot experiment, the four explants viz., apical shoot bud, middle leaf tip, leaf tip and spine (plate -2) of 1.5 cm. were subjected to the MS medium supplemented with different concentration of BAP + NAA for their response. Apical shoot bud, leaf tip, spine dose not showed callusing, where apical shoot bud multiplied. Showed multiple shoot induction without callusing. Further these responsive explants, were used for the standardization of protocol (plate-3). The size of explants at time of culture was found critical for shoot initiation. Similarly,
production of disease free planting materials using apical shoot bud have been reported by several work (Gupta et al., 2014), (kumara et al., 2015)

4.5.3 Effect of different concentration of plant growth regulators for shoot initiation.

Five different combinations of concentration of BAP + NAA were used in the study MS medium with 2mg/l BAP +.2mg/l NAA was found best for multiple shoot initiation for apical shoot bud used as they produced 5.0 mean number of shoots/ explant with 92.85% shoot initiation response. In the present study optimum response for shoot regeneration was obtained by combination of 2mg/l However, Ms media Supplemented with 4 mg/BAP+ 0.2 NAA also produce good number of multiple shoots with 3.69 mean number of shoots/ explants and the explants produced 86.66% response of shoot initiation (Table 4.1) BAP + 2mg/l NAA. The similar results were obtained by Kumari et. al. (2015), Ahmad et.al. (2007), Gupta et.al. (2014) and Mehta (2013). However, MS medium supplemented with 4mg/l BAP + 0.2mg/l NAA also produced good number of multiple shoot with 3.69 mean number of shoots/ explants.
Plate 4.1: Different explant used for inoculation.
Table 4.10: Effect different combination of hormones for shoot initiation

| Treatment code | Hormone combination | Number of explant inoculated | No. of culture contaminate | No. of explant response | No. of explants shoot initiated | No. of shoot initiated/plant Range | Mean | % Response |
|----------------|---------------------|------------------------------|---------------------------|------------------------|---------------------------------|----------------------------------|------|------------|
| T₁             | 3mg/l BAP+0.2 NAA   | 16                           | 1                         | 3                      | 12                              | 1-4                              | 2.6  | 80 %       |
| T₂             | 5mg/l BAP+0.2 NAA   | 16                           | 3                         | 2                      | 11                              | 2-3                              | 2.45 | 84.6%      |
| T₃             | 8mg/l BAP+0.2 NAA   | 16                           | 1                         | 5                      | 10                              | 1-2                              | 1.66 | 66.66%     |
| T₄             | 2mg/l BAP+0.2 NAA   | 16                           | 2                         | 1                      | 13                              | 5-7                              | 5.0  | 92.85%     |
| T₅             | 4mg/l BAP+0.2 NAA   | 16                           | 1                         | 2                      | 13                              | 2-5                              | 3.69 | 86.66%     |
Among other treatment except the treatments mentioned above using MS medium containing 0.2mg/l NAA in the combination with 3, 5, 8 mg/l BAP were found to be less responsive for apical shoot bud explants with mean 2.6, 2.45 and 1.66 mean number of shoots/ explant respectively under study. The concentration and combinations of hormones which showed highest response for shoot initiation was 2mg/l BAP+0.2 NAA. No. of explant shoot is 13 initiated in this media. Initiation media was 2mg/l BAP+0.2 NAA. Multiplication media is 1.5mg/l BAP+0.2NAA. 6 generation shoot multiplied.

4.5.4 Combination of plant growth regulator for on root initiation

For root induction, it was observed that basal MS medium devoid of phytohormones also induce rooting but percentage of rooting and number of roots per shoot were observed. 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. This produced good roots with 96% per cent rooting and 5.42 mean number of roots, whereas, 2.26 mean number of roots / shoot were obtained with 89.06% per cent rooting on half strength MS medium with 1.0 mg/l IAA + 500 mg/l activated charcoal. when apical shoot buds were culture using MS medium with 1.0 mg/l IBA +500 mg/l activated charcoal development of root was observed on explant. This result coincides with the findings of Shalini Mehta (2013) and Gupta et al. (2014).

4.5.5 Acclimatization of plants and transfer of regenerates to field

Regenerated plantlets of 6-8 weeks old with well developed roots were removed from culture vessels, washed thoroughly with the tap water to remove the Agar media. The roots were treated with 1% Bavistin for 10 min and transferred to sterilized cocopit in poly house. After 15 days transferred to sterilized earthen pots containing a mixture of sand, FYM , soil at a ratio of 1:2:1. The potted plants were kept in the net house for acclimatization before transfer to the open field. Plants were watered at two days interval. More than 95 per cent of the potted plants survived after one month of transfer and could be successfully transferred to the
field. The same media as that of polybag were used by Ahmed et.al. (2007) in their study.

In the present investigation, using different concentrations and different explants, it has been concluded that *aloe vera* can be successfully regenerated using *in-vitro* techniques, if the proper concentrations of plant growth regulators
Plate 4.2: Multiple shooting in explant of aloe vera.
Table 4.11a: Effect of different treatments on rooting response

| Treatment | Rooting hormones | No. of culture inoculated | No. of plant contaminated | No. of plant responded | No. of plant root responded | Mean plant Response in % |
|-----------|------------------|---------------------------|---------------------------|------------------------|-----------------------------|--------------------------|
| MS (half ) + 0.5 mg/l IBA + 500mg/l activated charcoal | 64 | 1 | 1 | 62 | 5.42 | 96.87% |
| MS (half ) + 0.5 mg/l IBA | 64 | 10 | 12 | 40 | 1.71 | 74.07% |
| MS (half ) + 0.5 mg/l IAA + 500mg/l activated charcoal | 64 | 2 | 9 | 53 | 2.26 | 84.12% |
| Basal MS (half) medium + 500 mg/l activated charcoal | 64 | 1 | 9 | 54 | 2.15 | 84.37% |
Table 4.11b: Effect of different treatments on rooting response

| Media                                      | Rooting percentage | Mean no. of roots/Explant | Type of root response                      |
|--------------------------------------------|--------------------|---------------------------|--------------------------------------------|
| Basal MS (half) medium +500 mg/l activated charcoal | 92.18%             | 2.15                      | Long thick root, Tap root.                 |
| MS (half) + 0.5 mg/l IBA + 500mg/l activated charcoal | 96.87%             | 5.42                      | Bunchy type, fibrous root.                 |
| MS (half) + 0.5 mg/l IAA + 500mg/l activated charcoal | 89.06%             | 2.26                      | Long few no. long thin type.               |
| MS (half) + 0.5 mg/l IBA                   | 87.5%              | 1.71                      | Bunchy type, thin root.                    |
Plate 4.3: Rooting response in shoot buds of aloe vera.
and initial explant tissues is made. The high frequency plant regeneration system for *in vitro* production of aloe vera through apical shoot bud explants has been developed during the present investigation. Various factors affecting the plant regeneration system has been examined and standardized and it opens avenues for large-scale production of genetically stable planting materials. Commercial micro propagation of aloe vera could be successfully performed utilizing apical shoot bud explants.
Plate 4.4: Completely developed plantlets and hardening of plants of aloe vera.
Aloe vera L. (Aloe barbadensis Mill, family Liliaceae) comprise of 500 species. It is cactus like succulent, perennial, xerophytic herbs with short stem and shallow root system growing in garden as well as in wild habitat. This plant is native of North America and has restricted distribution in our locality Raipur. It reproduces by vegetative methods through root suckers and adventitious shoot buds as the male flowers show sterility growth rate of these propagules is much slow. Aloe vera gel obtained from leaf pulp has been reported to have multiple beneficial properties for wound healing, including the abilities to penetrate and anesthetise tissues, arrests bacterial, fungal and viral growth, acts as an anti inflammatory agent and enhances blood flow.

Keeping the above fact, the present investigation was carried out to study the following objectives:

1. Screening of Aloe vera germplasm for high gel content.
2. To standardize protocol for high frequency in vitro regeneration of Aloe vera using various explants.
3. To study the effect of different combinations and concentration of plant growth regulators on large scale multiple shooting and establishment of plantlets.

Experiment was conducted in a Randomized Block Design with two set viz; in Set I, 13 germplasm Accession collected from different district of the C.G.

Germplasm accessions collected from three agro climatic zones of Chhattisgarh were evaluated for estimation of genetic variability, coefficient of correlation, path analysis and cluster analysis. Metric observation was recorded on three randomly selected competitive plants.
In present Investigation the PCV value was slightly higher than GCV which showed that the trait was influenced by the environment. Among the gel content per plant (g) and its attributing traits viz; number of suckers per plant had highest magnitude of GCV and PCV followed by number of spines per plant, plant height (cm), Leaf length (cm), Thickness of leaf (cm), No. of leaf/plant, Gel content/leaf(g). The high moderate GCV and PCV were observed for leaf width.

In present investigation high magnitude of heritability was recorded for most of the traits. The highest variability was recorded for the traits e.g. plant height (cm), leaf length (cm), no. of spines/plant, no. of suckers/plant and gel content. It indicate that these character is least influenced by the environment or selection of such character will be rewarded.

Some traits observed moderate genetic advance for trait plant height (cm), Leaf length (cm), Leaf width (cm), Thickness of leaf (cm), No. of spines/plant, No. of leaf/plant, no, of suckers and gel content (g).

All the traits possess high values of genetic advance indicate that the characters are governed by additive genes and selection will be rewarding for improvement of such trait.

High heritability coupled with high genetic advance as percentage of mean was found in the character gel content/plant. It indicates that heritability is due to additive gene effect or selection of such character may be effective. High heritability coupled with moderate genetic advance as percentage of mean was found in trait plant height (cm), leaf length (cm), No. of spine/plant, No. of suckers per plant. It showed that the character is govern by additive genes and selection will be rewarding for improvement of such trait.

Correlation coefficient analysis study indicated that gel content/plant(g) had positive and significant correlated with plant height(cm), leaf length (cm), leaf width (cm), thickness of leaf, no. of spines/leaf. may be advantageous for selection the high yield genotype in aloe vera from the available in gene pool.
Path coefficient analysis considering high gel content as dependent character revealed that No. of spine per plant showed highest positive direct effect followed by no. of leaf per plant followed by leaf width and leaf length. The other important show only negative direct effect on high gel content. Plant height showed highest negative effect followed by no. of suckers.

The maximum inter cluster distance was observed in between I and IV followed by cluster IV and III. This suggested that the hybridization programme involving parents from these cluster is expected to give higher frequency of better segregates or desirable combination for development of useful genetic stocks or varieties. The minimum inter cluster distance was observed in between II and III followed by cluster I and II.

The maximum intra cluster distance was observed in cluster IV followed by cluster I cluster II, cluster III.

The Accession no. 1 followed by Accession no. 12 and Accession no. 3 were the promising entries. The tissue culture Accession no. 1 was taken for mass multiplication in the present study.

Direct selection for plant height (cm), leaf length (cm) leaf width (cm), thickness of leaf (cm), no. of spines/leaf, no. of leaf /plant, may be advantageous for selection the high yield genotype in aloe vera from the available gene pool.

In a pilot experiment, the four explants viz., apical shoot bud, root tip, leaf tip and spine of 1.5 cm. were subjected to the MS medium supplemented with different concentration of BAP + NAA for their response. where apical shoot bud multiplied. Further these responsive explants were used for the standardization of protocol.

Among five different concentrations of BAP + NAA was used. MS medium with 2mg/l BAP +.2mg/l NAA was found best for multiple shoot initiation form apical shoot bud. The concentration and combinations of hormones which showed highest response for shoot initiation was 2mg/l BAP+0.2 NAA. No. of explant shoot is 13 initiated in this media. Initiation media was 2mg/l BAP+0.2 NAA. Multiplication media is 1.5mg/l BAP+0.2NAA. 6 generation shoot multiplied.

They produced 5.0 mean number of shoots/ ex plant with 92.85% shoot initiation response followed by MS medium supplemented with 4mg/l BAP +
0.2mg/l NAA also produced good number of multiple shoot with 86.66%. The best result was obtained by using MS medium with 0.5mg/l IBA +500 mg/l activated charcoal with 96% per cent rooting.

The roots were treated with 0.2% bavistin for 30 to 45 seconds and transferred to sterilized cocopit in poly house. After 1 week transferred to sterilized earthen pots containing a mixture of sand, FYM, soil at a ratio of 1:2:1. Plants survived after one month of transfer and could be successfully transferred to the field.

CONCLUSION

- In the present investigation, it was found that considerable variability was present in the experimental material under study.
- The gel content per plant (g) and its attributing traits, number of suckers per plant had highest magnitude of GCV and PCV followed by number of spines per plant, plant height (cm), Leaf length (cm), Thickness of leaf (cm), No. of leaf/plant, Gel content /leaf(g). The high moderate GCV and PCV were observed for leaf width (cm).
- The highest variability was recorded for the traits eg. plant height (cm), leaf length (cm), no. of spines/plant, no. of suckers/plant and gel content (g). It indicate that these character is least influenced by the environment or therefore selection of such character will be rewarded.
- Only some traits observed moderate genetic advance plant height(cm), Leaf length(cm), Leaf width(cm), Thickness of leaf(cm), No. of spines/plant, No. of leaf/plant, no of suckers and gel content(g).
- Correlation coefficient analysis study indicated that gel content/plant(g) had positive and significant correlated with plant height(cm), leaf length (cm), leaf width (cm), thickness of leaf, no. of spines/leaf. may be advantageous for selection the high yield genotype in aloe vera from the available in gene pool.
- Path coefficient analysis considering high gel content as dependent character revealed that No. Of spine per plant showed highest positive direct effect followed by no. of leaf per plant followed by leaf width and leaf length. The other important show only negative direct effect on high
gel content. Plant height showed highest negative effect followed by no. of suckers.

- The maximum inter cluster distance was observed in between I and IV followed by cluster IV and III. The minimum inter cluster distance was observed in between II and III followed by cluster I and II.

- The maximum intra cluster distance was observed in cluster IV followed by cluster I, cluster II ,cluster III

- Apical shoot bud multiplied. Further these responsive explants, were used for the standardization of protocol.

- MS medium with 2mg/l BAP + 0.2mg/l NAA was found best for multiple shoot initiation for meristem shoot. as they produced 5.0 mean number of shoots/ explant with 92.85% shoot initiation response.

- The best result was obtained by using MS medium with 1.0 mg/l IBA +500 mg/l activated charcoal. This produced good roots with 96% per cent rooting.

- The roots were treated with 0.2% bavistin for 30 to 45 seconds and transferred to sterilized cocopit in poly house. After 1 week transferred to sterilized earthen pots containing a mixture of sand, FYM, soil at a ratio of 1:2:1. Plants survived after one month of transfer and could be successfully transferred to the field. More than 95% of potted plant survived.
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## Appendix-A

Table: Weekly meteorological data for the crop period

| Wk. No. | Date       | Max. Temp. (°C) | Min. Temp. (°C) | Rainfall (mm) | Relative Humidity (%) | Wind Velocity (Kmph) | Evaporation (mm) | Sun Shine (hours) |
|---------|------------|-----------------|-----------------|---------------|-----------------------|----------------------|-----------------|-----------------|
| 26      | June       | 34.7            | 26.5            | 29.8          | 84.8                  | 5.2                  | 5.6             | 5.2             |
| 27      | 26-02 July | 34.8            | 26.2            | 32.4          | 88.2                  | 6.0                  | 6.1             | 7.5             |
| 28      | July       | 29.2            | 24.5            | 114.8         | 96.4                  | 11                   | 3.2             | 0.7             |
| 29      | 9-16       | 30.4            | 24.6            | 122           | 91.5                  | 8.0                  | 3.5             | 1.9             |
| 30      | 17-23      | 30.2            | 24.8            | 145.6         | 93.1                  | 5.4                  | 2.9             | 1.2             |
| 31      | 24-30      | 32              | 25.3            | 53.6          | 91.4                  | 4.2                  | 5.0             | 5.9             |
| 32      | 31-06 Aug  | 25.9            | 25.2            | 85.6          | 92.7                  | 7.3                  | 2.5             | 2.0             |
| 33      | Aug        | 29.3            | 22.1            | 51.8          | 89.2                  | 8.9                  | 9.0             | 2.1             |
| 34      | 7-13       | 28.7            | 21.4            | 11.6          | 88.2                  | 8.9                  | 2.6             | 2.0             |
| 35      | 14-20      | 31.7            | 25.6            | 26.4          | 92                    | 7.2                  | 4.4             | 4.1             |
| 36      | 21-27      | 32.5            | 25.9            | 53.8          | 90                    | 6.6                  | 4.3             | 3.7             |
| 37      | 28-03 Sep  | 30.5            | 25.2            | 44.6          | 86.2                  | 8.5                  | 5.0             | 1.1             |
| 38      | Sep        | 31              | 24.6            | 97.2          | 94.2                  | 7.8                  | 3.6             | 3.9             |
| 39      | 04-10      | 32.2            | 24.7            | 91.6          | 94.4                  | 6.9                  | 3.0             | 4.7             |
| 40      | 11-17      | 32.2            | 24.7            | 91.6          | 94.4                  | 6.9                  | 3.0             | 4.7             |
| 41      | 25-01 Oct  | 29.5            | 24.4            | 146.2         | 97                    | 88.1                 | 2.4             | 3.1             |
| 42      | Oct        | 30.7            | 24.6            | 45            | 96.2                  | 72.7                 | 2.3             | 3.1             |
| 43      | 02-08      | 31.9            | 22.3            | 0.8           | 92                    | 42.5                 | 1.3             | 3.6             |
| 44      | 09-15      | 31.3            | 18.7            | 0             | 91.1                  | 35.8                 | 1.1             | 3.9             |
| 45      | 16-22      | 31.4            | 18.4            | 0             | 86.5                  | 39.1                 | 1.6             | 3.7             |
| 46      | Nov        | 30.5            | 19.8            | 0             | 85.1                  | 49.1                 | 2.2             | 3.4             |
| 47      | 06-05 Dec  | 29.9            | 14.4            | 0             | 89                    | 26.7                 | 1.6             | 3.4             |

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| Date        | Range | Minimum | Maximum | Average | Standard Deviation | Minimum 5% | Maximum 95% |
|-------------|-------|---------|---------|---------|--------------------|------------|------------|
| Dec 06 – 12 |       | 28.33   | 12.3    | 88.29   | 33.57              | 1.34       | 2.79       | 7.34 |
| Dec 13-19   |       | 28.31   | 10.67   | 85.14   | 27                 | 2.1        | 3.67       | 8.67 |
| Dec 20-26   |       | 28      | 9.2     | 86.14   | 24.71              | 1.11       | 2.83       | 7.84 |
| Jan 27-02 Jan |     | 28.81   | 10.79   | 85.71   | 27.86              | 1.17       | 2.61       | 7.23 |
| Jan 3-9     |       | 28.49   | 12.03   | 89.14   | 32                 | 1.71       | 3.06       | 6.91 |
| Jan 10-16   |       | 26.23   | 10.7    | 85.14   | 74.29              | 1.89       | 2.96       | 7.01 |
| Jan 17-23   |       | 30.01   | 13.34   | 82.29   | 82.29              | 1.34       | 3.21       | 8    |
| Jan 24-30   |       | 29.31   | 13.57   | 83.57   | 83.57              | 1.9        | 3.46       | 7.99 |
| Jan 31-6 Feb|       | 31.37   | 12.29   | 81.29   | 33.86              | 1.36       | 3.97       | 9    |
| Feb 7 to 13 |       | 31.26   | 15.33   | 80      | 32.29              | 2.77       | 4.27       | 7.57 |
| Feb 14-20   |       | 32      | 15      | 80      | 23                 | 2          | 5          | 9    |
| Feb 21-27   |       | 33.89   | 15.4    | 66.86   | 16.14              | 2.6        | 6.17       | 10.21 |
| March 28-6 march |   | 35      | 17      | 67      | 21                 | 3          | 6          | 9    |
| March 7-13  |       | 31.94   | 18.59   | 70.71   | 31.29              | 3.64       | 5.17       | 6.9  |
| March 14-20 |       | 34.17   | 18.13   | 59      | 19.43              | 3.41       | 6.89       | 8.99 |
| March 21-27 |       | 37.89   | 20.4    | 62.57   | 13.57              | 2.27       | 7.06       | 9.11 |
| April 28-3 April |    | 41      | 24      | 54      | 13                 | 7          | 9          | 7    |
| April 4-10  |       | 42      | 25      | 38      | 16                 | 13         | 9          | 5    |
### Table: Mean performance gel content attributing characters

| accessions | plant height (cm) | leaf length(cm) | leaf width(cm) | thickness of leaf(cm) | no. of spines/plant | no. of leaf/plant | no. of suckers/plant | gel content/plant (g) |
|-------------|-------------------|-----------------|---------------|-----------------------|---------------------|-----------------|---------------------|-----------------------|
| acc.1       | 21.67             | 44.33           | 6.67          | 0.53                  | 32.67               | 11.33           | 6                   | 133.3                 |
| acc.2       | 33.33             | 43.33           | 6.43          | 0.6                   | 35.33               | 10.67           | 7.33                | 112                   |
| acc.3       | 34                | 41              | 6.03          | 0.77                  | 33.33               | 11.67           | 5                   | 122.4                 |
| acc.4       | 41                | 30              | 5.33          | 0.63                  | 26                  | 8               | 6.67                | 113.77                |
| acc.5       | 41.33             | 32              | 6.33          | 0.43                  | 29.33               | 12              | 6.67                | 37.93                 |
| acc.6       | 48                | 37              | 5.67          | 0.5                   | 19.33               | 9.33            | 6                   | 56.33                 |
| acc.7       | 49.33             | 25.67           | 5.33          | 0.47                  | 22.67               | 8.33            | 5.33                | 41.27                 |
| acc.8       | 51                | 31              | 6.33          | 0.53                  | 24.67               | 10.67           | 6.33                | 46.9                  |
| acc.9       | 52                | 32.67           | 6.33          | 0.53                  | 28.67               | 13.33           | 6.33                | 81.53                 |
| acc.10      | 52.67             | 25.67           | 5.33          | 0.5                   | 22.67               | 8               | 6                   | 37.07                 |
| acc.11      | 58                | 17.33           | 4             | 0.4                   | 14.67               | 6.67            | 5.67                | 21.2                  |
| acc.12      | 60.33             | 45.67           | 7.3           | 0.3                   | 42.67               | 15              | 7.33                | 128.27                |
| acc.13      | 61                | 42.33           | 4.67          | 0.77                  | 53.33               | 15              | 29                  | 71.27                 |
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