EFFECT OF DESICCATION ON CALLUS CULTURES IN COTTON

THESIS

Submitted to
Dr.Panjabrao Deshmukh Krishi Vidyapeeth, Akola
in partial fulfilment of the requirements
for the Degree of

MASTER OF SCIENCE
IN
AGRICULTURE
(AGRICULTURAL BIOTECHNOLOGY)

By

ZAMBRE SUPRIYA MANOHAR

BIOTECHNOLOGY CENTRE,
DEPARTMENT OF AGRICULTURAL BOTANY
POST GRADUATE INSTITUTE, AKOLA

DR. PANJABRAO DESHMUKH KRISHI VIDYAPEETH,
KRISHINAGAR PO, AKOLA (MS) 444104

Enrolment Number - BB/660

2009

DECLARATION OF THE STUDENT

I hereby declare that the experimental work and its interpretation of the Thesis entitled **"EFFECT OF DESICCATION ON CALLUS CULTURES IN COTTON"** or part their of has neither been submitted for any other degree or diploma of any University, nor the data have been derived from any thesis / publication of any University or scientific organization. The sources of material used and all assistance received during the course of investigation have been duly acknowledged.

Place: Akola
Date: 12/06/2009

Ku. Supriya Manohar Zambre
Enrolment No. BB/660

CERTIFICATE

This is certify that thesis entitled **"EFFECT OF DESICCATION ON CALLUS CULTURES IN COTTON"** submitted in partial fulfillment of the requirement for the degree of "Master of Science in Agricultural Biotechnology" of the Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola is a record of bonafide research work carried out by **Ku. Zambre Supriya Manohar** under my guidance and supervision.

The subject of the thesis has been approved by the Students Advisory Committee.

Place: Akola
Date : 12 / 06/ 2009

(Dr. E.R. Vaidya)
Chairman,
Advisory Committee

Countersigned

Associate Dean,
Post Graduate Institute, Akola
Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola.

THESIS APPROVED BY THE STUDENT ADVISORY COMMITTEE
INCLUIDING EXTERNAL MEMBER (AFTER VIVA-VOCE)

1. Chairman	Dr. E. R. Vaidya	
2. Member	Dr. A. A. Akhare	
3. Member	Dr. B.R. Patil	
4. Member	Dr. M.S. Dudhare	
5. Member	Shri. A.G. Deshmukh	
6. External Member	(Dr. M.P. Mohanil)	

ACKNOWLEDGEMENT

Success is not possible lonely without involvement of many minds and hands to beauty it. Emotions cannot be adequately expressed in words because then emotions are transformed into mere formalities. Nevertheless, Formalities have to be competed. My acknowledgement is many more than what I am expressing here.

I am immensely indebted to my honorable chairman, Dr. E.R. Vaidya, Associate Professor and Head of Section Agril. Botany, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola for his intellectual inspiration, valuable guidance, useful suggestions, constructive kind courtesy, and interest taken during the entire course of investigation. His constant and helpful suggestions were always a source of inspiration.

I have an immense pleasure in expressing my deepest sense of gratitude and humble indebtness towards Mr. A.G. Deshmukh, Assistant Professor and Dr. M.P. Mohril, Assistant Professor, Dr. A. A. Akhare Assistant Professor of Biotechnology Center, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola for their valuable guidance encouragement, important suggestion, kind help from time to time and sustained interest right from suggestion of problem to completion of thesis. I shall be ever grateful to them, because of their insightful guidance I could complete my research work.

I am duly acknowledged to Dr. B.R. Patil, Senior Research Scientist, Cotton Research Unit, Dr. PDKV, Akola as an advisory member and for timely supply of seed material.

It is my great pleasure to convey thanks to Dr. D.L. Sale, Associate Dean, Post Graduate Institute, Dr. PDKV, Akola and Dr. K.B. Wanjari, Head, Department of Agricultural Botany, Akola also I convey thanks to Dr. S.J. Gahurkar, In charge, Biotechnology Centre for extending necessary facilities to carry out the research work and constructive criticism during entire period of investigation without which this work could not have been possible.

I avail of this golden opportunity to express of my cordial thanks to Dr. D.B. Dhumale, Professor, Dr. P.L. Kulwal, Assistant Professor of

Biotechnology Center, Department of Agricultural Botany, Dr. PDKV, Akola for constructive criticisms.

I am extremely grateful to Mr. S. Munje, Assistant Professor, Biotechnology Centre and all staff members of Department of Agricultural Botany, Akola specially to Miss Abhilasha Kharkar (SRF) and Thawri sir for providing guidance and encouragement during research work.

I am thankful to Mr. Jirapure and Mr. Prashant of Tissue Culture Laboratory, Biotechnology Centre, Dr. PDKV, Akola.

Indeed the words are inadequate to express my indebtedness, gratitude reverence and love to my father Shri. Manohar A. Zambre and mother Sau. Meena M. Zambre. I find no such measures adequate to quantify all that my parents have done for me. I am also thankful to my brother Shyam and sister Prajakta and Uncle Associate Prof. Rajesh Ghorpade, Associate Prof. Archana Thorat and all family members for their hard jobs of educating me and shadowing me by showing their backs towards Sun, without which this work would not have seen the light of the day at all.

I am very much thankful to my friends Darshana, Madhuri, Dipika, Shilpa, Sonali, Shraddha, Sandesh,Valmik, Ganesh, Amol,Tushar, Jagdish, Ravi and all lab members .

No less in my gratitude to my Madam Mrs. Ganga Vaidya and her son Kapil whose support helped to overcome many difficulties during my studies.

I am also thankful to my junior colleagues Mr. Navid and Miss Priyanka for helping me in my research work.

My sincere thanks to all those well wishers and who have endured their cooperation directly or indirectly in completing this research works.

Place: Akola
Date : 12/06/09

(Ku. Supriya M. Zambre)
Enrolment No.BB/660

Table of Contents

(A) List of Tables

(B) List of Figures

(C) List of Plates

(D) Abbreviations

%	-	Per cent
/	-	Per
2, 4-D	-	2, 4- Dichlorophenoxy acetic acid
BAP	-	6-benzyleaminopurine
cm	-	centimeter
cv	-	Cultivar
Conc.	-	Concentration
et al.	-	and others
g	-	grams
h	-	hour/s
$HgCl_2$	-	Mercuric chloride
i.e.,	-	that is
IAA	-	Indole acetic acid
IBA	-	Indole buteric acid
KIN	-	Kinetin
l	-	litre
mg	-	milli gram
ml	-	milli litre
mM	-	milli molar
MS	-	Murashige and Skoog (1962) basal medium
NAA	-	Naphthalene acetic acid
No.	-	Number
°C	-	Degree centigrade
RH	-	relative humidity
Viz.,	-	namely
ZT	-	Zeatin
DAS	-	days after subculture

(E) GLOSSARY

Agar : A polysaccharide derived from certain algae and commonly used as a gelling agent in tissue culture media.

Aseptic culture : Raising cultures from a tissue or an organ after freeing it of bacteria, fungi and other microorganisms in an environment free of these microorganisms.

Autoclave : A vessel for sterilizing with steam under pressure.

Auxins : A class of plant growth hormones which cause cell elongation, apical dominance, root initiation etc. Indole acetic acid (IAA), naphthalene acetic acid (NAA), Indolebutyric acid (IBA) and 2,4-dichlorophenoxyacetic acid (2,4-D) are some of the auxins commonly used in tissue culture.

Biotechnology: Application of techniques to modify the fundamental processes of growth and reproduction in plants, animals and microorganisms, to enhance productivity or produce new products.

Callus : A tissue arising from disorganized proliferation of cells either in cultures or in nature.

Cell culture : Culture of single cells or small groups of similar cells.

Contaminants : Inthe present context, refers to microorganisms, which may inhibit the growth of cells or tissues in culture.

Culture : Growing cells, tissues, plant organs or whole plants in nutrient medium under aseptic condition e.g. cell culture, embryo culture, shoot tip culture, anther culture, etc.

Cytokinins : A class of plant growth hormones which are adenine (e.g. kinetin, benzilaminopurine, 2-isopentenyladenine, zeatin) or urea (e.g. thidiazuron) derivates and cause cell division, cell differentiation, shoot differentiation, breaking apical dominance, etc.

Embryo : An organized structure formed following a predetermined mode of development inside the female gametophyte with or without fertilization.

Embryogenesis : The process of embryo initiation and development either in the seed or in tissue cultures.

Explant : A plant organ or piece of tissue used to initiate culture.

Genotype : The genetic make up of an individual as determined by the set of genes carried in the chromosomes.

Growth regulators : Organic compounds other than nutrient that, in small amounts, influence growth, differentiation and multiplication, such as auxins, cytokinins, ethylene and gibberellin.

Hormones : Natural or synthetic chemicals that strongly affect growth, development or metabolism at very low concentrations i.e. cytokinins, auxins and gibberellins.

Hybrid : An organism resulting from a cross between genetically unlike parents.

Hypocotyl : The portion of seedling stem below the cotyledons and above the roots.

***In vitro* :** Literally `in glass'; now applied to any process carried out in sterile culture.

***In vivo* :** Literally `in life'; applied to any process occurring in a whole organism.

Induction: To cause initiation of a process or a structure.

Micropropagation : Asexual or vegetative propagation of plants *in vitro*.

Node : A region on the stem from where a leaf bearing and axillary bud arises.

Nutrient medium : A combination of nutrients and water, usually including several salts, a carbohydrate (e.g. sucrose) and vitamins, such a medium, liquid or gelled, is often referred to as a basal medium and may be supplemented with growth hormones and occasionally, with other defined and undefined substances.

Organogenesis: Differentiation of organs from cultured cells or tissue.

pH : A measurement of the degree of acidity or alkalinity on a scale of 1-14.

Photoperiod : The length of time plants are exposed to light in an alternating light dark interval sequence.

Polysaccharide: A group of carbohydrates composed of many units of various sugars.

Proliferation : Rapid multiplication of new units (cells, embryos, shoots etc.).

Regeneration: In tissue culture, a morphogenetic response that results in the production of new organs, embryos or whole plants from cultural explants or calli derived from them.

Somatic embryogenesis : In plant tissue culture, the process of embryo initiation and development from vegetative or non-gametic cells.

Stock solutions : Concentrated solutions from which portions are used to make media.

Subculture : With respect to plant tissue culture, this is the process by which the tissue or explant is first subdivided, then transferred into fresh culture medium.

Suspension culture : A type of culture in which cells or cell aggregates are cultured in liquid medium.

Transformation : A laboratory technique for genetic modification of plants by introducing one or few selected genes into cells to change their inherited characteristics.

(F) Thesis Abstract

a)	Title of the thesis	: **EFFECT OF DESICCATION ON CALLUS CULTURES IN COTTON**
b)	Full name of student	: **Zambre Supriya Manohar**
c)	Name and address of Major Advisor	: **Dr. E. R. Vaidya** M.Sc (Agri.) Ph.D. Associate Professor & Head of Section (Agri. Botany), College of Agriculture Dr. PDK V, Akola
d)	Degree to be awarded	: **M. Sc. (Agricultural Biotechnology)**
e)	Year of award of degree	: 2009
f)	Major subject	: Agricultural Biotechnology
g)	Total number of pages in the thesis	: 86
h)	Number of words in the abstract	: 321
i)	Signature of Student	:
j)	Signature, Name and address of forwarding authority	:

In- Charge,
Biotechnology Center,
Department of Agril. Botany.

Head
Department of Agril. Botany
Post Graduate Institute
Dr. PDKV, Akola.

Abstract

Cotton is one of the world's economically important fibre crops, and is extensively cultivated as an agro-industrial cash crop. Sometimes there is a

CHAPTER I
INTRODUCTION

1.1 Background information

Cotton "King of fibre", "White Gold" being the most important commercial crop, plays a key role in socio- economic affairs of the world. It finds its mention in the "Rigveda" the oldest scripture of the Hindus. Its antiquity has been traced to the 4th millennium B.C. Despite the increasing competition from artificial fibres the cotton fibre has maintained its pristine purity and importance to this day retaining its reputation.

Cotton is extensively cultivated as agro-industrial cash crop and also most important fibre crop amongst the fibre crops of the world. Cotton is predominantly self pollinated crop and often cross pollinated crop (Simpson, 1954). Cotton belongs to genus *Gossypium* which is one among eight genera under the tribe *Gossypieae* of family Malvaceae. This Dicotyledonous genus comprises approximately 50 species of which 45 are dipliod and five are allotetraploid (Fryxell 1992).The true lint bearing species of cotton are four, out of which two viz., *G. arboreum* and *G. herbaceum* are diploid (2n=26); commonly known as old World cotton or Deshi cotton in India, which are indigenous to Africa and Asia, and other two viz., *G. hirsutum* and *G. barbadense* L. are tetraploid (2n=52); commonly known as new World cotton or American cotton were initialy introduced in India during 17th and 18th A.D.

Cotton is big business with World production estimated at around 20 million tons. Cotton is playing a vital role in the economic, political and social affairs of the World. Chiefly a fiber crop, it has been estimated to contribute US $ 15-20 billion to the world's agriculture economy with over 180 million people depending on it for their livelihood (Benedict and Altman, 2001). Cotton is cultivated in about 70 countries of the World. The majority is produced by China, America, the Central Asian Republics, India, Pakistan, Brazil and Egypt. It provides 65 per cent raw

material to textile industry and contributed 1/3rd of total foreign exchange earning of India (Anonymous, 2006). India has a unique distinction of growing all the four spinnable lint bearing species of *Gossypium* and is one of the major producer of cotton sharing 20 per cent of world area of 93.73 lakh with production of 290 lakh bales and productivity of 562 kg lint/ha. In Maharashtra state, cotton is grown on an area of 31.33 lakh ha with production of 62 lakh bales and productivity of 336 kg/ha (CAB, 2008-09).

Vidarbha is the largest cotton growing region in Maharashtra state. It covers about 55 per cent area of Maharashtra state, which is 13.11 lakh ha with production 27 lakh bales with productivity of 350 kg lint/ha. The productivity of Maharashtra is 336 kg lint/ha (2007-08), which is lower than average productivity of India i.e. 526 kg lint/ha. (Anonymous, 2009)

Cotton has other more surprising uses from medicines and mattresses to seed oil and event sewage skins. This semi drying edible oil is used for salad or cooking oil and a non-oil residue is manufactured into a cotton cake for animal feed, fertilizer and paper. Cotton is grown chiefly for its fibre use. The production, consumption and trade of cotton based production further stimute the economy.

Regeneration of plants through tissue culture is an essential step in application of plant biotechnology for crop improvement. Cotton biotechnology hinges on two tightly interlaced processes transformation and regeneration. *Agrobacterium* mediated transformation and regeneration of cotton via somatic embryogenesis remains the preferred method of choice for generating transgenic cotton, as its advantages significantly outweigh the disadvantage relative to other methods (Wilkins *et al.*, 2000).

Plant regeneration has been achieved through organogenesis, somatic embryogenesis in *G. Klastchianum* but it failed to obtain complete plant. Plant regeneration in cotton through somatic

embryogenesis was reported by Davidonis and Hamilton (1983) in two years old calli derived from cotyledons. Since, then number of workers reported somatic embryogenesis and regeneration in cotton. However, most of successful regeneration reports were limited to Coker cultivar 201, 210, 312 and 350. *Agrobacterium* mediated method requires 10-12 months or longer to regenerate transgenic cotton plants (Firozabady *et al.*, 1987; Umberk *et al.*, 1987; Trolinder and Goodin, 1988a and 1988b; Lyon *et al.*, 1993; Thomas *et al.*, 1995), however somaclonal variation becomes problematic with such long culturing times (Stelly *et al.*, 1989; Firozabdy and DeBoer, 1993; Rajsekaran, 1996). Kumar and Pental (1998) reported regeneration of Indian cotton variety MCU-5 through somatic embryogenesis. Most of the reports indicated that regeneration response in cotton is highly cultivar dependent and Coker cultivar are highly responsive for regeneration but within the Coker cultivar there is seed to seed variation for regeneration (Gawel and Robacker, 1990; Trolinder and Xhixhian, 1989). Mathusamy and Jayabalan (2000) reported direct shoot on culture after 30 days on LRA-5166 genotype of *Gossypium hirsutum* by using leaf explant. Desiccation treatment was evaluated by Sakhanokho *et al.* (2004b) to improve the conversion efficiency of somatic embryo in diploid *Gossypium arboreum*.

Suprasanna *et al.* (2008) studied the partial desiccation for 6 h resulted in enhanced plant regeneration response from irradiated embryogenic cultures of sugarcane.

1.2 Importance of study

For transfer of desirable traits, such as fibre quality, resistance to insect, drought and diseases (Hutchinson *et.al.* 1947) the use of *in–vitro* regeneration protocol and desiccation treatment for improving the somatic embryogenesis would be more useful for the transformation technology.

Since onset of blooming in regenerated plants varies considerably seed production to produce genetically stable transgenic lines requires an additional 6-8 months, meaning that each transgenic plant may take two years or more to develop. In addition, cotton regeneration via somatic embryogenesis is highly genotype specific and highly generable lines selected from the absolute cultivar Coker 312 (Trolinder and Xhixian; 1989) serves as the industry stander at this time. Although linkage drags during introgression of transgene into elite cultivars continuous to be an issue of concern (Wilkins *et.al.* 2000).

1.3 Objectives of study

Keeping these considerations in view, the present investigation was conducted on "Effect of desiccation on callus cultures in cotton" with the following objectives,

i] To study the effect of desiccation on callus cultures, and

ii] To study the effect of desiccation on somatic embryogenesis in cotton.

The present study will be useful for developing regeneration protocol in popular cotton cultivar PKV-Rajat developed at Cotton Research unit, Dr. PDKV, Akola which is a prerequisite for transformation.

1.4 Scope and limitations of study

Callus culture study is helpful for *In-vitro* regeneration and which is basic need of genetic transformation in plants. Limited reports are available on regeneration in cotton. Despite of high commercial interest the success of biotechnological application in cotton has been limited due to genetic transformation. Major problem have been genotype dependence and low frequency of somatic embryogenesis making it difficult to regenerate plants from transgenic tissue. Therefore present study will be useful to standardize efficient regeneration protocol for transferring desirable genes.

The present study is limited to develop early stage somatic embryos in cotton, and it is needed to extend for complete regeneration of plants.

1.5 Hypothesis

The present study under taken to know the effect of desiccation on callus cultures and somatic embryogenesis in cotton cultivar PKV-Rajat developed by Cotton Research Unit, Dr. PDKV, Akola.

CHAPTER II

REVIEW OF LITERATURE

Cotton is one of the world's most economically important crop. *In vitro* cell culture techniques can provide plant breeders with new sources of genetic variation. Haploid plant production, protoplast fusion, gene transfer and exploitation of somaclonal variation techniques offer potential for crop improvement. Effective utilization of these produces ultimately hinges upon the development of efficient plant regeneration strategies.

Induction of somatic embryogenesis has been a significant achievement in plant tissue culture. Its application for mass production of plant genetic transformation and synthetic seed formation has been highlighted by Bajaj (1995). Somatic embryogenesis and plant regeneration are fundamental to the genetic improvement of cotton and desiccation treatment can also be incorporated into regeneration protocol to improved regeneration efficiency of *Gossypium* species (Sakhanokho *et al.* 2004b).

Plant tissue culture is defined as the process in which plant cell, tissue, organ or any plant part there of is grown aseptically in artificial environment (Scowcroft, 1984).

Desiccation is defined as the process of extracting moisture. Subjecting the cultured tissue to period of stress such as desiccation appears to improve the conversion of somatic embryos into mature embryos and subsequently to plants (Parrott *et al.* 1988., Yehosua *et at.* 1992).

Present review pertaining to effect of desiccation on callus cultures in cotton is presented in three parts,

2.1 General review of *in vitro* techniques

2.2 Callus induction and regeneration response in cotton.

2.3 Effect of desiccation an callus cultures and regeneration

2.3.1 Effect of desiccation on callus culture and regeneration in different plant species.

2.3.2 Effect of desiccation on callus culture and regeneration in cotton

2.1 General review on *in vitro* techniques

German botanist Gottlieb Haberlandt (1902) developed the concept of *in vitro* cell culture. He was first attempt to culture isolated, fully differentiated cells *in-vitro* on artificial media from leaves of *Lambium purpureum*. Although he failed in his goal to induce cell division, his work paved the way for development of plant tissue culture.

Winkler (1908) cultivated segment of string bean and observed some cell division but no proliferation. Kotte (1922) postulated that a true *in-vitro* culture could be made easier by using meristematic cell, such as root tip or bud. Effort to demonstrate totipotency led to development of techniques for cultivation of plant cell under controlled condition. This was made possible by the brilliant contributions form Gautheret (1939), Nobecourt (1939) and White (1939). Since then the technique of tissue culture has become an important tool for studying a wide range of basis and applied problem in biology.

The embryo culture technique was utilized by Laibach (1925) to recover hybrid progeny form an interspecific cross in *Linum species* Van Overbeek (1941) discovered the nutritional value of liquid endosperm of coconut for culture of isolated carrot embryo.

Skoog and Miller (1957) proposed the hypothesis that root-shoot differentiation in cultured Callus is regulated by auxin and cytokine ratio in the cultured medium.

The development of somatic embryo (embryoids) from carrot cells in suspension by Reinert (1959) in Germany and Steward (1958) in U.S.A. and subsequently from leaf mesophyll cells of *Malleaya cardata* by Kohlenbach (1966) were important discoveries of cell culture technology.

Morel and Martin (1952) recovered virus free Dahlia and potato plants from shoot meristem cultures to obtained disease free

The tissue and cell culture techniques provide new method for deriving variability and have a great potential for plant improvement, thus a promising tool for the plant breeders (Heinz *et al.*, 1979).

Regeneration through somatic embryogenesis is preferred over organogenesis because of a probable single cell origin of somatic embryos (Merkle *et al.*1995), thus reducing the chimeric transformation events.

Muthusamy and Jayabalan (2000) reported that the leaf bit explants derived from aseptically grown seven days old seedling of LRA-5166 were planted on MS+0.5mg/l NAA+ 0.1mg/l Kin produced direct shoot after 30 days of culture.

Hazra *et al.* (2002) was achieved plant regeneration from the seed derived decotyledonated split embryo axes of six Indian cotton cultivars viz., CNH-36, DCM-32, DHY-286, LRA-5166, LRK-516 and NHH-44 on MS medium.

Muthusamy *et al.* (2004) was obtained plantlets of *G. hirsutum* cv. MCU 5 and MCU 11 by using meristem shoot tip culture. The best shoot development was observed on media supplemented with 0.1 mg kinetin/ lit. Root development was observed on hormone free media with 0.3% cultivated charcoal.

2.2 Callus induction and regeneration response in cotton.

Callus induction and somatic embryogenesis are fundamental to cotton tissue culture biotechnology. However very few genotypes gave response to regeneration in cotton. In cotton somatic embryogenesis was first observed by Price and Smith (1979) in *G. khostzschianum* although complete plant could not be regenerated.

Royack *et al.* (1979) defined condition for suspension of two cotton G. hirsutum varieties IM 216 and Acal 44 which are resistant and susceptible, respective to the bacterial pathogen *Xanthomonus*

malvacearum (E.F.Sm) Dows A light friable callus were triedto induce root and shoot initiation were unsuccessful.

Davidonis and Hamilton (1983) subsequently described plantlet regeneration from a two-year-old callus culture of *G.hirsutum* var. Coker 310 through somatic embryogenesis. This procedure involved a lengthy culture period and was difficult to repeat.

Shoemaker *et al.* (1986) describe somatic embryogenesis and plant regeneration in *G.hirsutum* cvs. Coker 201 and 315.

Trolinder and Goodin (1987) defined the culture parameters for somatic embryogenesis in Coker varities and suggested the importance of genotype.

Trolinder and Goodin (1988) developed optimal media for induction of somatic embryogenesis from mature and immature tissue of *G. hirsutum* L. Cv. Coker 312. 100% somatic embryos was observed when culture grown on MS + 0.1 mg/l 2, 4-D + 0.5 mg/l KIN medium.

Trolinder and Xhixian (1989) screened 38 Gossypium genotype including *G.barbadense* accession for which they found a reduced level of embryogenesis compared with Coker 312.

Gawel and Robacker (1990) were tested three commercial varieties and three exotic accessions for ability to undergo somatic embryogenesis section of split petiole were cultured on three media and magnitude of embryogenesis was observed.

Firoozabady and DeBoer (1993) reported that a high 2ip: auxin ration is preferred for callus initiation and proliferation, but should be exchanged with a higher NAA: Cytokinin ration before diffentiation will occur.

Kalamani (1994) reported that hypocotyl explants of cv. MDU 9 when cultured on MS medium supplemented with 1 mg/L KIN + 1.5 mg IAA and formed callus in 20 days. The calli on subculturing to media containing BAP (2.0 mg\l) developed shoots. Multiple shoots were formed from each callus mass.

Hirimburegoma and Gamage (1994) cultured leaves, petioles, immature seeds explants of cotton cv. Coker 417 on basic MS medium supplemented with different growth regulators for 24 hours in light and dark. Callus formation was observed only on leaf and immature seed explants cultured in light with combination of 2,4-D and BAP. Callus from primary leaves was more homogeneous.

Rajasekaran (1996) observed successful regeneration of cotton (*G. hirsutum*) plants from cryopreserved embryogenic callus and cell suspension culture. Regenerated plants from cell suspension and embryogenic callus culture cryopreserved for more than four years exhibited normal morphological growth.

Rajasegar *et al.* (1996) observed the high frequency of callus induction and somatic embryogenesis on MS medium containing various concentrations and combinations of different growth regulators. Among the various explants, young leaves were found to be best for maximum frequency of callus induction on MS medium fortified with NAA (2.0mg/l) in combination with 2iP (3.0mg/l). These calli developed embryoids on MS medium containing 2iP and ABA (2.0mg/l) with 10mM glutamine. However plant regeneration frequency was low.

Gonzalez Benito *et al.* (1997) obtained callus from cotyledon and hypocotyl explants of *Gossypium hirsutum* race latifolium cv. CNPA Precoce-2 were cultured on MS media supplemented with 5 concentrations of 2,4-D and (2iP) either alone or in combination. Somatic embryos of different sizes and shapes appeared on MS medium supplemented with 2 g glutamine /l and no growth regulators. Plantlets were regenerated from these embryoids.

Cai-Xiao *et al.* (1997) cultured hypocotyls explants of cotton (*G. hirsutum*) cv. Simian 3, Glandless 2031 and Lumina 9554, on different medium. The medium containing 0.1 mg 2, 4-D, 0.1 mg IAA and 0.1 mg zeatin/l was optimum for initiation of callus from hypocotyls. Induction of embryogenic callus was achieved with 0.5 mg/l IAA and KIN.

Brar *et al.* (1998) established somatic callus tissue from immature embryos, hypocotyls, cotyledons, roots and young leaves of *G. arboretum* cv. LD-327, *G. herbaceum* cv. G. Cot.11, *G. hirsutum* cv. F-846, *G. thurberi* and *G. anomalum*. The best medium for callus initiation in diploid species was MS medium containing 0.1 mg 2,4-D + 0.1 mg KIN/l while in *G. hirsutum* MS medium containing 5.0 mg NAA + 0.1 mg KIN/l gave the best results. Among the different explants immature embryos and hypocotyls segments gave the highest amount of callus tissue, with younger explants giving better quality callus.

Kumar and Pental (1998) reported the protocol for the *in vitro* regeneration of Indian cotton cv. MCU-5 (*G. hirsutum*) Coker 201, 310, 315,312 somatic embryogenesis obtained from hypocotyl explant in 6-7 months. The highest frequency of regeneration has been reported in Coker varieties, however, even within Coker varieties there is seed to seed variation in regeneration.

Carvalho *et al.* (1998) evaluated the commercial cotton (*G. hirsutum*) cultivars CNPA process 2 and Coker 312 for somatic embryogenesis in different culture media. Calluses were induced in both varieties from hypocotyl pieces placed on MS medium supplemented with 0.1 mg 2,4-D + 0.5 mg (2ip) adenine /l.

Zhang *et al.* (1999) reported somatic embryogenesis and plant regeneration in five lines of low phenol content upland cotton (*G. hirsutum*). Grey soft and loose callus was initiated using hypocotyl segments cultured on medium containing MS inorganic and B_5 organic compounds, including kinetin and 2,4-D.

Guo-YuLong *et al.* (1999) cultured hypocotyl sections of the American variety Coker-312 and two other varieties of cotton (*G. hirsutum*) when cultured on 4 MSB based media to compare the intervarietal differences in callus induction and embryogenesis. On MSB medium containing 2, 4-D (0.05 mg/l) + kinetin (0.01 mg/l) or NAA (4.0 mg/l) +

kinetin (1.0 mg/l), callus induction rate was high for all the Coker varieties studied. Regenerated plantlets were obtained for all the Coker varieties.

Zhang *et al.* (2000) reported the regeneration protocol for elite cotton Variety Simian-3. The highest percent of callus induction on medium supplemented with Zeatin (3.0-5.0mg/L). Root was most responsive explant for production of somatic embryos the best medium for callus proliferation of embryogenic callus was MS+1.0mg/L2, 4-D+0.5mg/L Kin+0.5mg/L ZT and for diffentiation and germination of somatic embryos MS+ 0.1mg/L ZT+2gm/L activated charcoal. Plants could be regenerated within 60-80 days.

Sakhanokho *et al.* (2000) reported that callus was initiated from hypocotyl and cotyledon explants on a 1 mg/l KIN + 2 mg/l NAA. Friable embryogenic callus was selected and transferred on to MS with 0.1 mg/l KIN + 0.5 mg/l NAA. The selected callus was then transferred into a liquid embryo initiation medium. The liquid step was decreased by culturing time and increasing number of embryos per gram of cultured callus.

Zhang *et al.* (2001a) reported the direct somatic embryogenesis and plant regeneration in cotton (*G. hirsutum* cv. CCRI-12) on modified MS medium supplemented with 0.1 to 0.5 mg ZT/l, embryogenesis calluses and somatic embryos were directly induced with highest percentage induction (11.42%) at 0.1 mg/l ZT. The regenerated plants were transferred to soil.

Zhang *et al.* (2001b) reported the hypocotyls and cotyledon pieces of Coker-201 and CCRI-12 were placed on MS medium supplemented with 0.1 mg/l IAA + 0.1 mg/l ZT. The optimal medium for direct somatic embryogenesis was modified MS medium supplemented with 0.1 mg/l ZT + 2 g /l activated carbon. The somatic embryos were converted into normal plantlets when cultured on modified MS medium supplemented with 0.1 mg/l ZT. Plants could be regenerated within 60-80 days.

Nobre *et al.* (2001) described the development of a regeneration system from cotton stomata guard cells directly on epidermal strips for Coker-312 and 315. One fully developed plant was obtained from the culture of epidermal strips in both cultivars.

Sucheta Tripathy and Reddy (2002) reported the efficient plant regeneration from callus cultures of Indian cotton cultivars. Out of ten Indian cultivars, only six cultivars namely NCS-3, NA-920, NA-1325, Srisailam, LRA and PMC responded well in the plant regeneration. Only the MS media supplemented with 2 mg/l BA + 2 mg/l IAA was the best for plant regeneration.

Suresh Kumar *et al.* (2002) studied six *G. hirsutum* lines of diverse origin with two explant viz. hypocotyls and cotyledons for induction of callus. On an average over genotypic, explant and media response, highest number of callus induction on media MS+ O.1MG/L +24D+ 0.5mg/l kinetin. None of genotype showed differentiation from callus.

Xue-Meifeng *et al.* (2002) reported the embryogenic calluses of *G. hirsutum* cv. Chuan-239 and examined the changes in colour, growth and embryogenic capability during long-term subculture. Embryogenic capability can be maintained for a long time of subculture on MSB medium containing 0.1 mg Kin/l + 1.9 g KNO_3/lit and on MSB supplemented with 0.1 mg KIN/l + 0.1 mg IAA/l. The duration of embryogenic callus subculture was recommended not to exceed 1.5 years.

Sun YuQiang *et al.* (2003) reported the high frequency somatic embryogenesis and plant regeneration from hypocotyl explant of *G. klotzschianum* Anderss. Embryogenic culture were induced on MSB medium with 0.9 mM 2, 4-D + 2.32 mM KIN. Somatic embryos cultured on MSB medium with PGR to form secondary embryo and embryo developed into normal plantlets on PGR- free MSB medium.

Kumria *et al.* (2003) initiated the embryogenic calluses, from hypocotyl or cotyledonary leaf sections on MS medium containing 0.1 mg/L 2,4-D + 0.5 mg/L KIN + 3% maltose. More than 70% of cotyledonary

embryos developed into normal plantlets when cultured on MS medium containing 0.05-mg/L gibberellic acid.

Suresh Kumar *et al.* (2002) screened twelve cotton genotypes belonging to 4 cultivated cotton species adapted to different agro climatic conditions for *in vitro* regeneration Diploid species gave higher response for *in vitro* dedifferentiation than tetraploids. Hypocotyls explants showed higher response than cotyledons for callus induction.

Mishra *et al.* (2003) suggested a step towards genotype independent regeneration of cotton (*G. hirsutum* L.) by selection for regeneration potential (RG) in commercial seed of elite cultivars. Based on the number of fertile plants regenerated on a percent seedling basis, RG was estimated as 17.4%, 44.4% and 80% in Acala cotton cultivars `Maxxa', `Ultima' and `Riata', respectively.

Aydn *et al.* (2004) developed a highly reproducible system for efficient somatic embryogenesis to regenerate plantlets from cotton (*G. hirsutum* L.) cv. (Nazilli-M-503 and Nazilli-143). Shoot apices, hypocotyls and nodes explants are used. High frequency of embryogenic calli was initiated on MS medium with 1 g/l PVP + 1 mg/l BAP + 0.5 mg/l KIN for Nazilli M-503 and 1 mg/l PVP, 2 mg/l BAP + 0.5 mg/l KIN for Nazilli-143.The germination frequency of somatic embryo (42.9%) for Nazilli-M-503 and (22-30%) for Nazilli-143.

Sakhanokho *et al.* (2004 a, b) tested 15 elite upland cotton lines from southeast germplasm. Eight lines developed by Georgia Agriculture experiment station and seven by the USDA-ARS per Dec cotton breeding programmed. Three Pee Dee Lines, PD 97019, PD 97021 and 97100 and one Georgia line, GA 98033 responded to at least one of the three medium treatments. The regeneration efficiency of the Georgia and Pee Dee lines was relatively low as compared with standard Coker 312 cultivar.

Hussain *et al.* (2004) reported an efficient *in vitro* plant regeneration system to produce somatic embryos using immature zygotic

embryos in cotton (*G. hirsutum* cv. C-312 JS). Rapid callogenesis was observed in these immature zygotic embryos. Upon the development of roots and leaves, plantlets were potted in a mixture (1:1:1) of sand, silt and peat moss under high humidity then hardened under greenhouse condition. Using this system, they were able to regenerate approximately 70% of healthy plantlets.

Kouadia *et al.* (2004) established cultures of two cultivars of cotton ISA-205 N and ISA GL7 cultivated in café `d' Ivoire from hypocotyls fragments. These fragments were excised from etiolated and non-etiolated seedlings (absence or presence of chlorophyll). The non-etiolated hypocotyls of both cultivars showed significantly higher percentage of callus induction than etiolated ones. It was shown that the darkness pre-treatment of cultured explants would be good condition to induce somatic embryogenesis of cotton.

Kumar and Tuli (2004) reported the increase in somatic embryogenesis efficiency and induction of developmental synchrony in embryogenic callus culture of cotton by single cycle of myo-inositol depletion in liquid culture. Friable callus were collected over filler mesh 40 and subjected to one cycle of myo-inositol starvation. This induced a highly synchronized embryogenesis in the culture.

Ikram-Ul-Haq and Zafar (2004) used hypocotyls explants for induction of callus through culturing on MS 1 a medium supplemented with 2,4-D and KIN. The high frequency of globular embryogenesis was achieved when well proliferating callus was cultured on MS (1/2 strength) medium for four weeks. Complete plants were regenerated through somatic embryogenesis from hypocotyls explants within 6 months.

Sakhanokho *et al.* (2005) reported the improvement in somatic embryogenesis in several cotton lines (*G. hirsutum* L.) from Georgia and Pee Dee germplasm with culture media containing various putrescine concentrations. The best results were obtained with NAA as compared to 2; 4-D based culture medium. Inclusion of 0.5 mg/l

putrescine improved somatic embryo induction for most treatments and lines tested.

Efe (2005) cultured 3,9,15 and 21 days old ovules excised from 2 different cotton species (*G. hirsutum* cv. Ersan 92 and *G. barbadense* cv. Agdas-21) on MS medium supplemented with various ingredients. To determine the effects of growth regulators on callus formation, 0.5, 1.5, 2.5, 3.5 mg/l IAA and 0.5, 1.5, 2.5, 3.5 mg KIN/l were added to the media in 16 combinations. Callus formation was 85.2% and 44.1% for *G. hirsutum* and *G. barbadense* respectively.

Ikram-Ul-Haq (2005) reported the callus proliferation was considered best on MS-1a (2.0 mg/l NAA; 0.1 mg/l ZT; 0.1 mg/l KT) when 6 weeks old callus from MS-1b (0.1 mg/l, 2,4-D; 0.5 mg/l KT) medium, there is no need to select embryogenic calluses for somatic embryogenesis, as all of them were converted to somatic embryos. When cotyledonary embryo cultured on MS with 0.05 mg/l GA3 medium were developed into normal plantlets and rooted simultaneously.

Rao *et al.* (2005) reported that wild cotton species can contribute a valuable gene pool for agronomically desirable cultivated tetraploid cultivars. Different callus induction media were tested with varying concentrations of hormones. Different stages of embryogenicity such as early torpedo stage, late torpedo stage; heart stage, globular stage and cotyledonary stage were observed in wild relatives of cotton.

Verma *et al.* (2005) reported the highest callus induction frequency (85.4%) in genotype H-771 on MS 63 medium (MS basal medium containing 0.1 mg/l ZT and 3% maltose) using hypocotyls.

2.3 Effect of desiccation on callus cultures.

2.3.1 Effect of desiccation on callus cultures and regeneration in different crop species.

Partial desiccation treatments have been reported to be beneficial for embryogenesis and plant regeneration in several plant

species. Water deficit, directly through partial desiccation, is also known to stimulate ethylene evolution (Guinn1976) that may influence morphogenetic response *in vitro*. Partial desiccation has been reported to enhance somatic embryo differentiation and development in soybean (Hammatt and Davey 1987), grapevine (Gray 1987), Cassava (Mathews et al 1993), banana (Srinivas et al 2006).

Carman (1986) improved somatic embryogenesis in wheat by partial desiccation.

Saito *et al.* (1991) have reported dehydration of the culture through filter paper improved aeration of the culture, there by probably reducing ethylene level, which might have been helpful.

Tsukahara and Hirosawa (1992) reported that dehydration of suspension culture derived calli of the Japonica variety Susanishiki enhanced the regeneration frequency from 5% up to 47%.

The desiccation treatment showed improvement in the conversion of somatic embryos into healthy plants as compared to plant obtained from the non-desiccated embryogenic calli. Slow desiccation cause stimulation of loss of turgor and substantial accumulation of starch and protein reserve in comparison with no or rapid dehydration (Etienne *et al.* 1993)

Rance *et al.* (1994) obtained 66.5%, 61.1% and 73.7% of calli that regenerated plants for the indica rice varieties TN 1, IR 72, and IR 64 by partial desiccation treatment where as in non desiccated controls only 30.0%, 15.5% and 18.7% of calli regenerated respectively.

Vertucci and Forrant (1995) and Haslekas *et al.* (1998) reported that during desiccation, free radical scavengers may provide additional protection because the development of desiccation tolerances which involves a period of water stress coincides with an increase in free radical scavengers in seeds.

Jain *et al.* (1996) also reported that 24hr partial desiccation increases three time of shoot regeneration in indica rice varities, Basmati 385 and Pusa basmati-1.

Jain *et al.* (1996)[illegible]suggested the improvement in regeneration potential has been suggested to be due to decreased water contented in dehydrated callus leading to an increase in endogenous abscisic acid level. Higuchi and Maedu (1990) and improved of oxygen supply to the callus.

Azria and Bhalla (2000) studied the plant regeneration from mature embryo derived callus of Australians rice (*Oryza sativa* L.) varieties. Partial desiccation resulting in up to 20% loss of fresh weight of callus significantly increased the regeneration frequency.

Chand and Sahrawat (2000) carried out partial desiccation of embryogenic calli prior to transfer to regeneration medium and observed increased regeneration frequency of desiccation treatment to callus culture of cv. Safari-17 and cv. Kasturi.

Desai *et al.* (2004) demonstrated that partial desiccation treatment of embryogenic callus improved plant regeneration in sugarcane.

Saharan *et al.* (2004) reported 48 hr. desiccation treatment was enhance shoot regeneration from 63 to 82% in indica rice cv. HKR-48 and HKR-125 on MS modified medium supplemented with 2 mg/lit Kin + 0.5 mg/l NAA + 30 mg/l sucrose + 6 g/l gelrite followed by MS + 1 mg/l 2 ip+ 30g/lit sucrose + 6 g/l gelrite.

Wagiran (2008) reported the plant regeneration in Japonica rice when 48 hr desiccated calli were transferred into optimal shoot regeneration media contained MS + 3 mg/l kinetin + 0.5 mg/l NAA + 3 mg/l gelrite. The regeneration frequency was highest at 76, 70 and 30% in variety Nipponbare, Hayohishikin and Fujisaka 5 respectively.

Suprasanna *et al.* (2008) reported that to stimulate regeneration response, irradiated cultures were subjected to partial

desiccation for 6 h and the treatment resulted in enhanced plant regeneration response.

Deng *et al.* (2009) reported that in four genotypes of maize calluses regenerated shoot best after 48 h of desiccation corresponding to a 23.7% average desiccation percentage and regeneration frequency reached 42.74% which increase 1.5 – 2.1 fold compared with that of non desiccated calluses.

2.3.2 Effect of desiccation on callus culture and regeneration in cotton

Voo *et al.* (1991) reported somatic embryogenesis and plant regeneration in *G.hirsutum* L. cv. coker 312 with the help of chemical and physical desiccation treatment.

Kumaria *et al.* (2003) used hypocotyls and cotyledon explants for initiation of embryogenic calli on MS medium containing 0.1mg/l2, 4 D, 0.5 mg/l Kinetin and 3% maltose produced globular- stage somatic embryos. However the frequency of globular embryos developing into normal plantlets improved considerably when culture on filter paper placed on MS medium. About 33% of globular embryos developed into cotyledonary embryos and subsequently to normal plantlets.

Sakhanokho *et al.* (2004) reported the 3-days desiccation treatment best and can be incorporated into regeneration protocol to improve the regeneration efficiency of cotton .The medium MS+0.2M glucose+ 2.6μM NAA + 0.2μM kin underwent a 3-d desiccation treatment 49 percent of these immature somatic embryos were converted into plantlets, in diploid cotton variety A-29 (PL 529722).

These improved result will helped to pave the way for future genetic transformation and can also be incorporated into regeneration protocol to improved regeneration efficiency of *Gossypium* species.

CHAPTER III

MATERIAL AND METHODS

The present *investigation was carried on* "Effect of desiccation on callus cultures in cotton *(G. hirsutum* L.)" at Plant Tissue Culture Laboratory, Biotechnology Center, Department of Agricultural Botany, Post Graduate Institute, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola.

3.1 Material

3.1.1 Genotypes

The experimental material of present investigation comprised of one popular variety of cotton (*G. hirsutum* L.) viz., PKV-Rajat. The seeds were obtained from Cotton Research Unit, Dr. PDKV, Akola.

3.1.2 Explants

3.1.2.1 Raising seedling in aseptic media

Explants were obtained from aseptically grown seedling at different age were used for present investigation.

Following two explants were obtained from four- five days old seedlings,

i. Hypocotyl segments

ii. Leaf bits

3.1.3 Glasswares

All the glasswares used were of 'BOROSIL' brand. For nutritional studies, culture bottles and culture tubes were used. For preparation of media and other work, Erlenmeyer flasks, beakers, measuring cylinders, petridishes, pipettes etc. were used.

3.1.4 Equipments

Equipments like LAF (Laminar Air Flow) cabinet, autoclave, growth chamber, B.O.D. incubator, weighing balance, hot air oven and pH meter were used.

3.1.5 Disinfectant and sterilizers

The chemical required for disinfection and sterilization of glasswares are given in Table 3.1.

Table 3. 1. Chemicals required for disinfection and sterilization

Particulars	Name of chemical	Conc. required
Glasswares	Potassium dichromate dissolved in conc. H_2SO_4	200 g Potassium dichromate per lit H_2SO_4
Surfactants	Mercuric chloride Ethanol	0.1% (w/v) 70% (v/v)

3.1.6 Washing glasswares

Glasswares were disinfected in solution containing potassium dichromate and concentrated H_2SO_4. To prepare the solution 60 g of pot dichromate was dissolved in 35 ml of distilled water and was heated for 2-3 minutes and final volume was made to one litre by adding conc. H_2SO_4. Glasswares were immersed for overnight in this solution. The following day, glasswares were thoroughly washed in tap water then with distilled water. They were allowed to dry on draining racks.

3.1.7 Sterilization of glasswares

Culture bottles, culture tubes were plugged with non-absorbent cotton and covered with butter paper. Petridishes, pipettes, beakers etc. were autoclaved at 1.5 p.s.i. pressure at 121^0C for an hour. They were then dried in hot air oven at 80-100^0C for two hours.

3.2 Culture media

3.2.1 Medium used for culture studies

In the present study, MS (Murashige and Skoog, 1962) medium was used. Modifications were made in this medium by adding suitable auxins and cytokinins.

3.2.2 Preparation of stock solutions

3.2.2.1 Basal media

All stock solutions were prepared in sterilized doubled distilled water, poured in well stopper bottles and stored in refrigerator. The constituents of MS media are given in the Table 3.2.

Table 3.2. Composition of Murashing and Skoog (1962) basal medium.

Ingredients	Chemical formula	Qty. (mg/l)	Conc. of stock solutions taken per litre medium
Major nutrients (stock A)			
Ammonium nitrate	NH_4NO_3	1650	50 ml
Potassium nitrate	KNO_3	1900	
Calcium chloride	$CaCl_2.2H_2O$	440	
Magnesium sulphate	$MgSO_4.7H_2O$	370	
Potassium dihydrogen orthophosphate	KH_2PO_4	170	
Minor nutrients (stock B)			
Potassium iodide	KI	0.83	5 ml
Boric acid	H_3BO_4	6.20	
Manganese sulphate	$MnSO_4.4H_2O$	22.30	
Zinc sulphate	$ZnSO_4.7H_2O$	8.60	
Sodium molybdate	$Na_2MoO_4.2H_2O$	0.25	
Copper sulphate	$CuSO_4.5H_2O$	0.025	
Cobalt chloride	$CoCl_2.6H_2O$	0.025	

Iron source (Stock C)			
Sodium EDTA	Na_2 EDTA	37.80	
Ferrous sulphate	$FeSO_4.7H_2O$	27.80	5 ml
Vitamin (stock D)			
Myoinositol		100	
Nicotic acid		0.5	
Pyridoxine HCl		0.5	5 ml
Thiamine HCl		0.1	
Glycine		2.0	

3.2.2.2. Hormones

Auxins like IAA, IBA were dissolved in few drops of NaOH and 2, 4-D in few drops of ethanol. They were heated slightly and gradually diluted to the required volume with double distilled water. Cytokinins like BAP and kinetin were dissolved in a few drops of 1 N NaOH, heated slightly and gradually diluted to required volume with double distilled water.

3.2.2.3. Carbohydrate sources

High quality sucrose was used at the concentration of 3%.

3.2.2.4. Gelling agent

i) Agar of LR grade at a conc. of 8 g per litre used for reparation of media.

ii) Phytagel (Sigma U.S.A.) at conc. of 3g per litre used for preparation of media.

3.2.3. Preparation of medium

The basal medium used for the present study was MS (Murashige and Skoog, 1962). The details of ingredients of MS media are given in Table 3.2.

For the preparation of one litre of media 50 ml of stock A solution was transformed to a clean, sterile, conical flask. To the same container 5 ml each of stock B, C and D solutions were transferred and stirred well. Growth regulators viz., IAA, 2,4-D BAP and kinetin were added to solution as per the requirement. The sucrose used at a concentration of 30 g/l (3%) or glucose was used at a conc. of 30g/l (3%) or 20g/l (2%), dissolved in water and added to the medium. The volume of solution was made up to one litre.

The pH of the medium was adjusted to 5.8. Then gelling agent such as agar or phytagel was added to the medium at 0.8 percent or 0.3 percent concentration and medium heated to dissolve the gelling agent without any clods. The medium was poured into previously sterilized bottles. The quantity of media per bottles was 30 ml. The bottles or cultures tubes were autoclaved at 121^0C at 15 lbs $inch^{-2}$ for 20 minutes.

3.2.4. pH of the medium

The pH of the medium was adjusted to 5.8 by adding 0.1 N HCl or 0.1 N NaOH before adding the solidifying agent.

3.3. Methodology

All aseptic manipulations including surface sterilization inoculation and transfer were carried out in a Horizontal Laminar Air Flow (LAF) cabinet fitted with an ultraviolet tube, which was turned on for half an hour before use. Before use of the cabinet it was smeared with ethyl alcohol (70%). All the cultures were incubated at 25 ± 2^0C under 16 hours of light (2000 Lux) and 8 hours of dark period.

3.3.1 Delinting:

Seeds were treated with commercial sulphuric acid for 5 to 10 s and then washed the seed with water and poured the seed in to lime solution for 5 min to remove excess of sulphuric acid; the seed were washed under running tap water for 10 min and dried. Before the start of experiment germination of seed was checked this is about 90 %.

3.3.2 Surface sterilization:

Delinted seed were surface sterilized by keeping the seeds water for half and hour then washed with detergent for three minute, followed by washing the seed with distilled water for three minute. Seeds were treated with 0.1% mercuric chloride for 10 minute. Seed were rinsed thrice in large volume of sterile distilled water prior to imbibitions and germination.

3.3.3 Seed germination:

Seed were germinated on full strength solid MS medium at 28±2°c with 16 hours light (1000 Lux) and eight hours dark period.

3.3.4 Explants culture:

Hypocotyls and leaf bits explants were obtained from 4 and 5 days old seedling aseptically raised on full strength MS media. Hypocotyls section of 4 mm length and leaf bits 3 mm lengths were cultured on MS medium supplemented with growth regulators.

3.3.5 Callus Induction:

To initiate the callus, explants were excised from surface sterilized seed and placed on to MS medium supplemented with growth regulators. After 4 weeks of incubation, primary callus were transferred to the fresh callus multiplication media for subculturing. Embryogenic callus was selected and maintained by subculturing once in every four week for

six month. Callus induction and subculture occurred at 25±2°C and in darkness.

The different treatment combinations of auxins and cytokinins concentrations with basal medium used for callus induction are given in Table 3.3. These were formulated on the basis of available literature.

Table 3.3. Combinations of auxins and cytokinins with basal media used for callus induction

Sr.No.	Treatment	MS (1962) basal		
		NAA mg /l	BAP mg/l	IAA mg/l
1	CM-1	3.00	0.00	0.00
2	CM-2	3.00	0.00	0.50
3	CM-3	3.00	0.00	1.00
4	CM-4	3.00	0.30	0.00
5	CM-5	3.00	0.50	0.00
6	CM-6	5.00	0.00	0.00
7	CM-7	5.00	0.00	0.50
8	CM-8	5.00	0.00	1.00
9	CM-9	5.00	0.30	0.00
10	CM-10	5.00	0.50	0.00
11	CM-11	10.0	0.00	1.0

3.3.6 Callus multiplication and proliferation.

The different treatment combinations of auxins and cytokinins concentrations with basal medium used for callus multiplication and proliferation are given in Table 3.4.

Table 3.4. Combinations of NAA and BAP with basal medium used for callus multiplication and proliferation in cotton

Sr. No.	Treatment	MS (1962) basal	
		NAA mg/l	BAP mg/l
1	M-1	5.00	
2	M-2	5.00	0.50
3	M-3	5.00	1.00
4	M-4	5.00	1.50

Observations were recorded with respect to response of two explants in different treatments combinations for

i. **Days required for callus initiation:** No. of days for initiation of callus were recorded after apparently observation of callus formation and counted in days from inoculation.

ii. **Percent callusing:** It was recorded at 45 days after inoculation of explants with one subculture at 21 days after inoculation. The frequency of callus induction was expressed in terms of percentage.

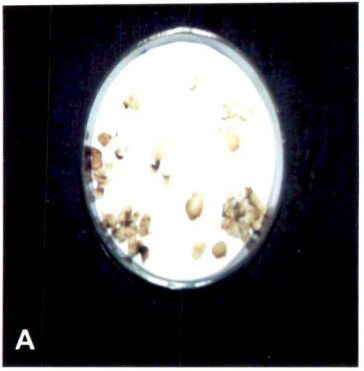
iii. **Relative growth rate of callus (%):** It was recorded at 30 DAS of 100mg callus on multiplication and proliferation medium.

3.3.7 Desiccation treatment:

Desiccation of callus was carried out at one and six month old sub cultured and was replicated in three times. Calluses around (5 gm) were treated with following two-desiccation agent and four different time duration interval under aseptic condition.

i. Filter paper 2hr, 4hr, 6hr, 8hr.

ii. Silica gel 2hr, 4hr.6hr, 8hr.



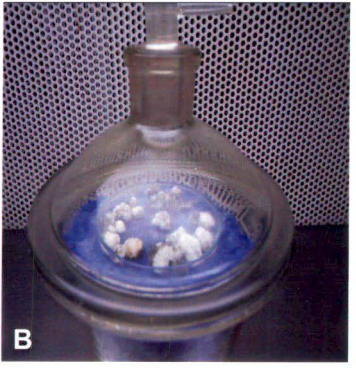


Plate 1 : Desiccators used for desiccation of callus
A - Filter paper
B - Silica gel

i) Filter paper:

Callus was carried out at one year and at six months old of subcultured and was replicated three times. Callus around five grams per treatment were transferred on to sterilized empty Petri dishes containing two dry sterilized whatman-1 filter paper. Petri dishes were sealed with Parafilm and kept at 25± 2°C in dark for 2,4,6,8 hours respectively.

ii) Silica gel:

About 5 gram per treatment of callus was desiccated using silica gel in sterilized desiccator for 2,4,6,8 hours and after desiccation, callus was transferred to regeneration medium.

Observation were be recorded after desiccation treatment

i) **Relative growth rate of callus on wt basis after desiccation:** It was recorded at 30 DAS of 100 mg desiccated callus on multiplication and proliferation medium.

a. **Fresh weight of callus (g):** Fresh weight of callus was recorded before each desiccation treatment.
b. **Weight of callus after desiccation (g):** Weight of callus after desiccation was recorded after each desiccation treatment.
c. **Moisture loss after desiccation treatment:** We used the term desiccation percentage to indicate the percent loss in callus fresh weight before and after desiccation.

$$\text{Desiccation percent (\%)} = \frac{A - B}{A} \times 100$$

Where,

A= Fresh weight of callus

B= Weight of callus after desiccation

ii) Regeneration response:

By comparing the effect different desiccation treatment, regeneration response for control calluses and desiccated calli with different media combination were recorded.

3.3.8 Somatic embryogenesis

Well-grown calli from different explants were cultured on MS medium supplemented with different levels of auxins and cytokinins. The treatment combinations of auxins and cytokinins concentrations used for regeneration study are given in Table 3.5. These were formulated on the basis of available literature (Trolinder and Goodin, 1988a and b; Kumar Shashi and Pental, 1998; Zhang *et al.* 2001; Suresh Kumar *et al.* 2002; Sakhanokho *et al.* 2004 and Verma *et al.* 2005).

Table 3.5. Combinations of auxin and cytokinins with basal medium used for somatic embryogenesis in cotton

Sr. No	Treatments	Auxin (mg/l)				Cytokinin (mg/l)	
		2,4-D	NAA	IAA	IBA	BAP	Kinetin
1	SM-1	0.0	0.0	0.0	0.0	0.0	0.0
2	SM-2	0.1	0.0	0.0	0.0	0.0	0.5
3	SM-3	0.1	0.0	0.0	0.0	0.0	1.0
4	SM-4	0.1	0.0	0.0	0.0	0.0	1.5
5	SM-5	0.2	0.0	0.0	0.0	0.0	1.0
6	SM-6	0.2	0.0	0.0	0.0	0.0	1.5
7	SM-7	0.2	0.0	0.0	0.0	0.0	2.0
8	SM-8	0.4	0.0	0.0	0.0	0.0	0.4
9	SM-9	0.5	0.0	0.0	0.0	1.0	0.0
10	SM-10	0.5	0.0	0.0	0.0	1.5	0.0
11	SM-11	0.5	0.0	0.0	0.0	2.0	0.0
12	SM-12	1.0	0.0	0.0	0.0	1.0	0.0

Contd..

Table 3.5 contd.

13	SM-13	1.0	0.0	0.0	0.0	1.5	0.0
14	SM-14	1.0	0.0	0.0	0.0	2.0	0.0
15	SM-15	0.0	0.1	0.0	0.0	0.1	0.0
16	SM-16	0.0	0.1	0.0	0.0	0.5	0.0
17	SM-17	0.0	0.1	0.0	0.0	1.0	0.0
18	SM-18	0.0	0.1	0.0	0.0	1.5	0.0
19	SM-19	0.0	0.1	0.0	0.0	0.0	1.5
20	SM-20	0.0	0.25	0.0	0.0	1.0	0.0
21	SM-21	0.0	0.4	0.0	0.0	0.0	0.4
22	SM-22	0.0	0.5	0.0	0.0	0.1	0.0
23	SM-23	0.0	0.5	0.0	0.0	0.5	0.0
24	SM-24	0.0	0.5	0.0	0.0	1.0	0.0
25	SM-25	0.0	0.5	0.0	0.0	1.5	0.0
26	SM-26	0.0	0.5	0.0	0.0	2.0	0.0
27	SM-27	0.0	0.75	0.0	0.0	1.0	0.0
28	SM-28	0.0	1.0	0.0	0.0	0.2	0.0
29	SM-29	0.0	1.0	0.0	0.0	0.5	0.0
30	SM-30	0.0	1.0	0.0	0.0	1.0	0.0
31	SM-31	0.0	1.0	0.0	0.0	1.5	0.0
32	SM-32	0.0	1.0	0.0	0.0	2.0	0.0
33	SM-33	0.0	1.0	0.0	0.0	3.0	0.0
34	SM-34	0.0	1.5	0.0	0.0	0.0	0.2
35	SM-35	0.0	1.5	0.0	0.0	0.0	0.5
36	SM-36	0.0	1.5	0.0	0.0	0.0	1.0
37	SM-37	0.0	1.5	0.0	0.0	0.0	1.5
38	SM-38	0.0	1.5	0.0	0.0	0.0	2.0
39	SM-39	0.0	2.0	0.0	0.0	0.0	0.1
40	SM-40	0.0	2.0	0.0	0.0	0.0	0.5

Contd..

Table 3.5 contd.

41	SM-41	0.0	2.0	0.0	0.0	0.0	1.0
42	SM-42	0.0	2.0	0.0	0.0	0.0	1.5
43	SM-43	0.0	2.0	0.0	0.0	0.0	2.0
44	SM-44	0.0	2.0	0.0	0.0	0.5	0.0
45	SM-45	0.0	2.0	0.0	0.0	0.75	0.0
46	SM-46	0.0	2.0	0.0	0.0	1.5	0.0
47	SM-47	0.0	2.0	0.0	0.0	2.0	0.0
48	SM-48	0.0	2.5	0.0	0.0	0.5	0.0
49	SM-49	0.0	0.0	0.0	0.0	0.5	0.0
50	SM-50	0.0	0.0	0.0	0.0	1.0	0.0
51	SM-51	0.0	0.0	0.0	0.0	1.5	0.0
52	SM-52	0.0	0.0	0.0	0.0	2.0	0.0
53	SM-53	0.0	0.0	0.0	0.0	0.0	0.2
54	SM-54	0.0	0.0	0.0	0.0	0.0	0.5
55	SM-55	0.0	0.0	0.0	0.0	0.0	1.0
56	SM-56	0.0	0.0	0.0	0.0	0.0	1.5
57	SM-57	0.0	0.0	0.0	0.0	0.0	2.0
58	SM-58	0.0	0.0	0.0	0.0	0.5	0.5
59	SM-59	0.0	0.0	0.0	0.0	1.0	1.0
60	SM-60	0.0	0.0	0.0	0.0	3.0	0.0
61	SM-61	0.0	3.0	0.0	0.0	0.5	0.0
62	SM-62	0.0	3.0	0.0	0.0	1.0	0.0
63	SM-63	0.0	3.0	0.0	0.0	1.5	0.0
64	SM-64	0.0	3.0	0.0	0.0	2.0	0.0
65	SM-65	0.0	4.0	0.0	0.0	0.5	0.0
66	SM-66	0.0	4.0	0.0	0.0	1.0	0.0
67	SM-67	0.0	4.0	0.0	0.0	1.5	0.0
68	SM-68	0.0	4.0	0.0	0.0	3.0	0.0

Contd.

Table 3.5 contd.

69	SM-69	0.0	5.0	0.0	0.0	0.5	0.0
70	SM-70	0.0	5.0	0.0	0.0	1.0	0.0
71	SM-71	0.0	5.0	0.5	0.0	0.0	0.0
72	SM-72	0.0	0.0	0.1	0.0	0.1	0.0
73	SM-73	0.0	0.0	0.1	0.0	0.5	0.0
74	SM-74	0.0	0.0	0.1	0.0	1.5	0.0
75	SM-75	0.0	0.0	0.1	0.0	2.0	0.0
76	SM-76	0.0	0.0	0.2	0.0	0.0	0.0
77	SM-77	0.0	0.0	0.5	0.0	0.5	0.0
78	SM-78	0.0	0.0	0.5	0.0	1.5	0.0
79	SM-79	0.0	0.0	0.5	0.0	2.0	0.0
80	SM-80	0.0	0.0	1.0	0.0	0.5	0.0
81	SM-81	0.0	0.0	1.0	0.0	1.5	0.0
82	SM-82	0.0	0.0	1.0	0.0	2.0	0.0
83	SM-83	0.0	0.0	1.5	0.0	0.5	0.0
84	SM-84	0.0	0.0	1.5	0.0	1.0	0.0
85	SM-85	0.0	0.0	1.5	0.0	1.5	0.0
86	SM-86	0.0	0.0	1.5	0.0	2.0	0.0
87	SM-87	0.0	0.0	2.0	0.0	0.0	0.5
88	SM-88	0.0	0.0	2.0	0.0	0.0	1.0
89	SM-89	0.0	0.0	2.0	0.0	0.0	1.5
90	SM-90	0.0	0.0	2.0	0.0	0.0	2.0
91	SM91	0.0	0.0	0.1	0.5	0.0	0.0
92	SM-92	0.0	0.0	0.1	1.0	0.0	0.0
93	SM-93	0.0	0.0	0.1	1.5	0.0	0.0
94	SM-94	0.0	0.0	0.1	2.0	0.0	0.0
95	SM-95	0.0	0.0	1.0	0.5	0.0	0.0
96	SM-96	0.0	0.0	1.0	1.0	0.0	0.0

Contd..

Table 3.5 contd.

97	SM-97	0.0	0.0	1.0	1.5	0.0	0.0
98	SM-98	0.0	0.0	1.0	2.0	0.0	0.0
99	SM-99	0.0	0.0	2.0	0.75	0.0	0.0
100	SM-100	0.0	0.0	2.0	1.0	0.0	0.0
101	SM-101	0.0	0.0	2.0	1.5	0.0	0.0
102	SM-102	0.0	0.0	2.0	2.0	0.0	0.0
103	SM-103	0.0	0.0	0.0	0.0	2.0	0.5
104	SM-104	0.0	0.0	0.0	0.0	2.0	1.0
105	SM-105	0.0	0.0	0.0	0.0	2.0	1.5
106	SM-106	0.0	0.0	0.0	0.0	2.0	2.0
107	SM-107	0.0	10	0.0	0.0	1	0.0
108	SM-108	0.0	10	0.0	0.0	1.5	2.0
109	SM-109	0.0	10	0.0	0.0	0.0	0.0
110	SM-110	0.0	10	0.0	0.0	0.0	2.5

3.4 Statistical analysis

For recording observations on induction of callus, five aliquots of each treatment were used. For recording observation of regeneration of callus also, three aliquots were taken.

The mean and standard error were calculated as per procedure given by Panse and Sukhatme (1958).

$$X = \frac{\Sigma Xi}{N}$$

$$SD = \sqrt{\frac{\Sigma (Xi - X)^2}{N}}$$

$$SE = \frac{SD}{\sqrt{N}}$$

Where, $\bar{X}$ = Arithmetic mean

X_i = Sum of i^{th} observation

N = Number of observations

SE = Standard error

SD = Standard deviation

CHAPTER IV
RESULTS AND DISCUSSION

The present investigation was conducted on "Effect of desiccation on callus cultures in cotton". The experimental material comprised of two explants viz., hypocotyls and leaf bits of popular cotton (*G. hirsutum* L.) variety PKV– Rajat. The media treatments were formulated by using different combinations of auxins and cytokinins with Murashige and Skoog (1962) as basal medium. The observations were recorded on different aspects and the results are presented in this chapter.

4.1 Callus induction in cotton.

4.1.1 Days required for callus initiation.

The effect of different 11 media treatments for initiation of callus using two explants, hypocotyls and leaf bits of cotton variety PKV-Rajat was studied and the data are presented in Table 4.1 and Fig. 1.

It was found that minimum numbers of days (11.95±0.10) were required for initiation of callus from hypocotyls in PKV-Rajat and in leaf bits (24.34± 0.22) in CM-10 and CM-11 medium, respectively. Numbers of days required for initiation of callus were less at higher concentration of auxins as against lower concentration of auxin in both the explants.

In medium CM-2, rhizogenesis was observed within 10 to 15 days and secondary roots were also developed. For leaf bits there was higher percentage of rhizogenesis than hypocotyl. These results were comparable to earlier report given by Muthusamy and Jayabalan (2000). Callus initiation was maximum from hypocotyls within 10-11 days as compared to leaf bits. Texture of callus in leaf bits was loose friable and white in colour. Callus developed from hypocotyls was compact in texture

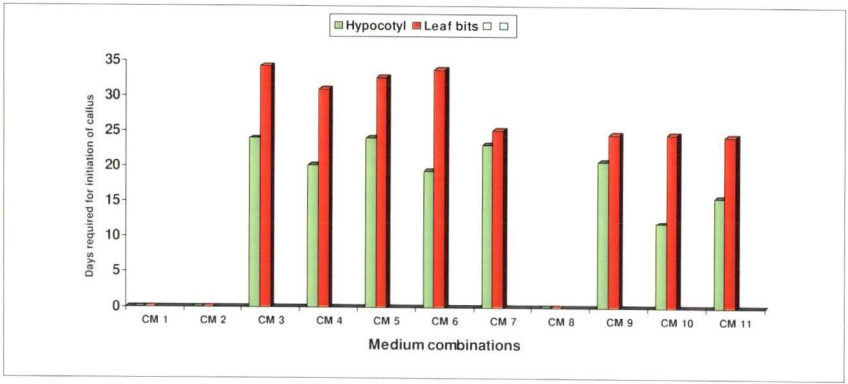


Fig. 1 Days required for initiation of callus in cotton on different medium combinations by using hypocotyls and leaf bits as explants.

similar results were also reported for hypocotyls in cotton (*Gossypium hirsutum*) by Shoemaker *et al.* (1986) and Verma *et al.* (2005).

Overall performance of hypocotyls was found to be more responsive than leaf bits for initiation of callus on different media combinations. Difference for callus initiations using different explants were also observed by Verma *et al.* (2005), Zhang *et al.* (2001) in cotton. Genotypic differences for callus initiation were also observed by Shoemaker *et al.* (1986) and Trolinder and Xhixhian (1989) in cotton.

Table 4.1 Days required for callus initiation in cotton

Sr. No.	Medium	Explants	
		hypocotyls	Leaf bits
1	CM-1	No callus	No callus
2	CM-2	Rooting	Rooting
3	CM-3	24.02±0.04	34.28±0.38
4	CM-4	20.21±0.11	31.07±0.07
5	CM-5	24.06±0.11	32.71±0.11
6	CM-6	19.34±0.01	33.84±1.34
7	CM-7	23.13±0.11	25.17±0.18
8	CM-8	No callus	No callus
9	CM-9	20.73±0.09	24.65±0.08
10	CM-10	11.95±0.10	24.70±0.13
11	CM-11	15.63±0.06	24.34±0.22

4.1.2 Percent callus induction in cotton

The effect of 11 treatments on percent callusing from two explants viz., hypocotyls and leaf bits of PKV-Rajat was studied and the data are presented in Table 4.2 and Fig.2.

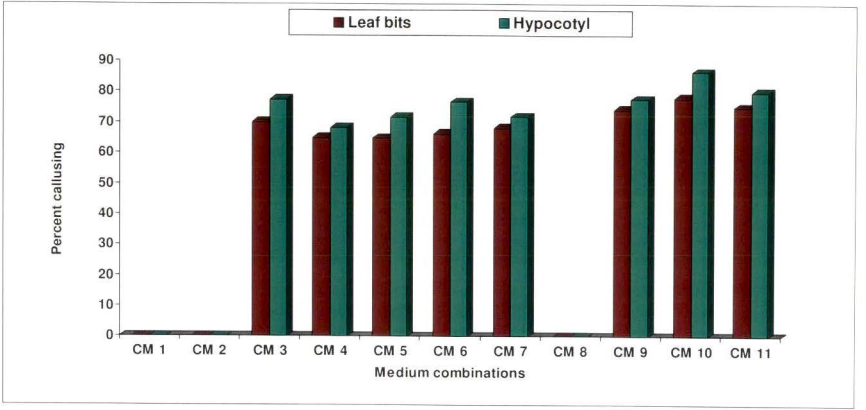


Fig. 2 Percent callus induction in cotton on different medium combinations by using leaf bits and hypocotyls as explants

Hypocotyls and leaf bits explants gave higher percent callusing on medium CM-10 and CM-11 having combination (5mg/l NAA+0.5mg/l BAP and 10mg/l NAA +1mg/l BAP). Similar results were also reported for hypocotyls by Suresh Kumar *et al.* (2003); Zhang *et al.* (2001) and Verma *et al.* (2005).

Table 4.2: Percent callus induction in hypocotyls and leaf bits explants of cotton.

Sr. No.	Medium	Explants	
		Hypocotyls	Leaf bits
1	CM-1	No callus	No callus
2	CM-2	Rooting	Rooting
3	CM-3	77.42	70.07
4	CM-4	68.10	64.91
5	CM-5	71.68	64.69
6	CM-6	76.54	66.25
7	CM-7	71.73	68.08
8	CM-8	No callus	No callus
9	CM-9	77.46	74.23
10	CM-10	86.40	78.00
11	CM-11	79.94	74.96

4.2 Callus multiplication and proliferation in cotton.

After callus induction, the whitish brown callus was transferred on callus multiplication and proliferation media. The growth rate of callus obtained from hypocotyls and leaf bits on weight basis was recorded 30 DAS and estimated in percentage in PKV-Rajat. The results are presented in Table 4.3.

Four media were tried for callus multiplication, out of which, M-3 media (5mg/l NAA and 0.5mg/ BAP) was found to be better for multiplication and proliferation of embryogenic callus. The basal media with higher concentration of NAA and lower concentrations of BAP were found to be suitable for proliferation of embryogenic callus than NAA alone. The present results were comparable to Brar *et al.* (1998).They reported (MS + 5.0 mg/l NAA + 0.1 mg/l KIN) to be the best for callus proliferation and maintenance.

The callus multiplied and proliferated faster on M-3 than other medium at 30 DAS of callus. However, friable white embryogenic callus was produced on M-1 which is primary criteria for somatic embryogenesis. These results are in agreement with results of Zhang *et al.* (2001).

Table 4.3: Callus multiplication and proliferation in cotton

Sr.No	Medium	Explant	
		Hypocotyls	Leaf bits
1	M-1	164.90±0.10	165.74±0.31
2	M-2	127.33±0.34	122.05±0.41
3	M-3	243.83±0.50	230.22±0.11
4	M-4	187.64±0.29	172.98±0.11

4.3 Effect of Desiccation on callus growth in cotton

The effect of ten media treatment on fresh weight and weight of callus after desiccation from two explants, hypocotyls and leaf bits of

each desiccation treatment in PKV Rajat were studied and the data are presented in Table 4.4, 4.5, 4.6, 4.7, and 4.8.

4.3 .1 Effect of desiccation on relative growth rate of callus

After desiccation treatment of callus for different time interval (2, 4, 6 and 8 hr) using two desiccators viz., silica gel and filter paper, the desiccated callus was transferred on callus multiplication and proliferation media. Observations were recorded for growth rate of callus on weight basis and estimated in percentage of callus multiplication 30 DAS for both explants viz., hypocotyls and leaf bits of cotton cultivar PKV-Rajat. The results are presented in Table 4.4.

The maximum growth rate of callus was observed from hypocotyls (451.33 $\pm$ 1.13) in eight hours of desiccation period using filter paper as desiccators on medium M-2 followed by (440.00 $\pm$ 0.54) medium M-3.

Two media treatments were tried out of which M2 medium was found to be better for multiplication and proliferation of desiccated callus. In desiccated callus, maximum percentage of callus multiplication than control was observed. Differences were also observed between desiccators; in silica gel desiccated callus multiplication and proliferation was lesser than filter paper desiccated callus.

Similar results were also observed by Deng *et al.* (2009) and suggested filter paper desiccation in maize. They observed that increased desiccation period resulted in increase in callus multiplication rate.

Table 4.3 Relative growth rate of callus on weight basis after desiccation treatment

Medium	Desiccation Period (hrs)	Explants	Percent increase of callus 30 DAS after desiccation (%)	
			SG	FP
M-2	Control (Without desiccation)	Hy	262.67±0.87	
		LB	261.67±3.15	
M-3		Hy	255.00±0.72	
		LB	261.67±3.15	
M-2	2	Hy	324.33±0.96	330.33±0.42
		LB	316.33±0.63	324.33±0.96
M-3		Hy	316.67±0.56	334.67±1.13
		LB	314.33±0.87	323.67±0.57
M-2	4	Hy	388.67±0.83	410.58±0.83
		LB	384.33±0.57	402.00±0.72
M-3		Hy	482.67±0.68	414.23±0.42
		LB	378.33±0.57	390.0±0.54
M-2	6	Hy	411.67±0.96	441.67±1.29
		LB	397.33±0.68	433.67±0.8
M-3		Hy	402.33±0.68	435.60±0.82
		LB	393.33±0.68	425.33±0.68
M-2	8	Hy	421.33±0.96	451.33±0.13
		LB	416.67±0.23	442.67±0.68
M-3		Hy	411.00±0.54	440.00±0.54
		LB	406.67±0.13	425.67±0.31

HY-hypocotyls

LB –leaf bits

DAS- days after sub culture

4.3.2 Effect of two hours desiccation on callus growth

The effect of two hour desiccation using silica gel and filter paper on callus culture from two explants viz., hypocotyls and leaf bits of PKV-Rajat were studied (Table 4.5)

In case of old callus of hypocotyls, maximum weight of callus (88.48 + 0.23) was recorded with silica gel desiccation whereas in new callus it was (90.84 ± 0.24) on M4 medium.

In case of old callus of leaf bits, maximum weight (88.49 ± 0.56) was observed with silica gel desiccation on M2, whereas in new callus it was (90.68 ± 0.09) on M4 medium.

Maximum weight of callus after desiccation of hypocotyls using filter paper was 57.41 ± 0.24 whereas in new callus it was (57.45 ± 0.04) on medium M1.

Maximum weights after desiccation of old and new calli of leaf bits using filter paper was (56.58 ± 0.19) and (55.95 ± 0.22), respectively on medium M1.

In general, new callus loose higher moisture percentage as compared to old callus during desiccation. Between two desiccation, faster rate of desiccation was observed with filter paper than silica gel.

In case of silica gel, higher desiccation percentage was recorded from new callus of hypocotyls (11.78) and leaf bits (12.51) on M1 medium, whereas in case of filter paper highest desiccation percentage was recorded from old callus of hypocotyls (44.53) and leaf bits (44.92) on M2 medium. These results were also comparable to Suprasanna *et al.* (2008) in sugarcane and Rance *et al.* (1994) in rice. They dried calli sufficiently to loss 10% to 40% of their fresh weight.

Table 4.5 Effect of two-hour desiccation on callus weight in cotton

Sr. No.	Medium	Desiccators	Weight of callus after desiccation in percentage				Desiccation percentage			
			Hypocotyls		Leaf bits		Hypocotyls		Leaf bits	
			Old	New	Old	New	Old	New	Old	New
1	M1	SG	88.22 ±0.17	89.85 ±0.11	87.32 ±0.39	90.06 ±0.04	11.78	11.20	12.68	12.51
		FP	57.41 ±0.24	57.45 ±0.04	56.18 ±0.06	55.95 ±0.22	42.59	43.22	43.82	44.81
2	M2	SG	86.94 ±1.37	89.22 ±0.15	88.49 ±0.56	87.49 ±0.06	11.52	11.45	11.51	10.82
		FP	55.47 ±0.30	56.78 ±0.25	56.58 ±0.19	54.34 ±0.12	44.53	43.69	43.42	44.92
3	M3	SG	83.81 ±2.3	86.11 ±1.4	88.37 ±0.99	89.18 ±0.15	11.37	11.48	11.63	11.37
		FP	56.04 ±0.21	56.05 ±0.09	54.84 ±0.24	55.94 ±0.17	43.96	43.06	43.09	44.73
4	M4	SG	88.48 ±0.23	90.84 ±0.24	88.11 ±2.7	90.68 ±0.09	11.38	11.25	11.89	11.96
		FP	56.16 ±0.11	56.31 ±0.21	54.11 ±0.18	54.75 ±0.07	43.84	43.36	44.92	43.82

4.3.2 Effect of four-hour desiccation on callus weight in cotton

The effect of four-hour desiccation using silica gel and filter paper on callus culture from two explants viz., hypocotyls and leaf bits of PKV-Rajat were studied (Table 4.6).

In case of old callus of hypocotyl maximum weight (82.62 ± 0.35) was recorded using silica gel desiccated calli on M2 medium, whereas in new callus it was (81.99 ± 0.00) on M3 medium.

In case of old callus of leaf bits, maximum weight (82.84 ± 0.31) was observed using silica gel desiccated calli on M1 medium, where as in new callus it was (83.19 ± 0.16) on M4 medium.

Maximum weight of callus after desiccation of hypocotyls using filter paper was (52.13 ± 0.18) where as in new callus it was (57.45 ± 0.04) on M2 medium.

Maximum weight of callus after desiccation of old callus of leaf bits using filter paper was (53.06 ± 0.22) and in new callus it was (52.82 ± 0.11) on M3.

In case of silica gel, higher desiccation percentage was recorded from new callus of hypocotyls (19.36) and leaf bits (18.71) on M4 medium, whereas in case of filter paper, highest desiccation percentage was recorded from old callus of hypocotyls (49.82) and leaf bits (49.00) on M2 and M1 media respectively. These results are comparable to Diah and Bhalla (2000). In Australian rice variety, they observed partial desiccation resulting up to 20% loss of fresh weight of callus.

Table 4.7. Effect of four-hour desiccation on callus growth in cotton.

Sr. No.	Medium	Desiccators	Weight of callus after desiccation in percentage				Desiccation percent			
			Hypocotyls		Leaf bits		Hypocotyls		Leaf bits	
			Old	New	Old	New	Old	New	Old	New
1	M1	SG	81.85± 0.12	81.14 ±0.42	82.84 ±0.31	80.56 ±0.41	18.15	18.86	17.16	17.61
		FP	52.11± 0.02	50.68 ±0.35	51.00 ±0.20	52.38 ±0.01	47.89	48.71	49.00	48.79
2	M2	SG	82.62± 0.35	80.24 ±0.04	82.58 ±0.45	83.19 ±0.16	17.38	18.01	17.42	17.47
		FP	52.13± 0.18	51.05 ±0.47	53.06 ±0.22	52.32 ±0.18	49.82	48.66	48.27	49.40
	M3	SG	82.28± 0.38	81.99 ±0.00	82.3± 00.28	82.98 ±0.18	17.72	19.32	17.70	18.06
		FP	52.06± 0.14	51.29 ±0.07	52.75 ±0.11	52.82 ±0.11	47.94	48.14	46.94	47.62
4	M4	SG	80.64± 0.65	80.68 ±0.22	81.29 ±0.30	80.66 ±0.73	19.36	18.90	18.71	17.02
		FP	50.97± 0.04	51.34 ±0.07	51.21 ±0.10	51.43 ±0.24	49.03	49.16	47.25	47.68

4.3.3 Effect of six hours desiccation on callus weight in cotton

The effect of six-hour desiccation using silica gel and filter paper on callus culture by from two explants viz., hypocotyls and leaf bits of PKV-Rajat was studied (Table 4.7).

In case of old callus of hypocotyls, maximum weight (79.74± 0.01) was recorded using silica gel desiccated callus on M3 medium, whereas in new callus it was (78.59 ± 0.13) on M1 medium.

In case of old callus of leaf bits, maximum weight (78.28 ± 0.30) was observed using silica gel desiccated callus on M1 medium, where as in new callus it was (78.66 ± 0.52) on M4 medium.

Maximum weight of callus after desiccation of old callus of hypocotyls using filter paper was (47.85 ± 0.18) whereas in new callus it was (48.08 ±0.26) on M2 medium.

Maximum weight of callus after desiccation of old callus of leaf bits using filter paper was (47.99 ± 0.17) and in new callus it was (47.80 ± 0.61) on medium M2.

In case of silica gel, higher desiccation percentage was recorded from new callus of hypocotyls (22.85) and in leaf bits (23.00) on M3 and M4 media, whereas in case of filter paper, highest desiccation, percentage was recorded from old callus of hypocotyls (52.91) and leaf bits (53.78) on M2 and M1 media, respectively. These results obtained are in agreement with the finding of Suprasanna *et al.*, (2008) in sugarcane.

Table 4.7. Effect of six hours desiccation on callus weight in cotton.

Sr No.	Medium	Desiccators	Weight of callus after desiccation in percentage				Desiccation percent			
			Hypocotyls		Leaf bits		Hypocotyls		Leaf bits	
			Old	New	Old	New	Old	New	Old	New
1	M1	SG	78.93 ±0.43	78.59 ±0.13	78.28 ±0.30	77.59 ±0.59	21.1	22.4	21.7	23.0
		FP	47.85 ±0.18	48.08 ±0.26	47.33 ±0.20	46.22 ±0.41	52.2	52.7	52.6	53.7
2	M2	SG	78.11 ±0.22	77.52 ±0.49	76.08 ±0.14	77.00 ±0.95	21.9	22.3	22.8	22.0
		FP	46.67 ±0.19	47.28 ±0.07	47.99 ±0.17	47.80 ±0.16	53.3	52.9	52.0	52.2
3	M3	SG	79.74 ±0.01	77.70 ±0.34	77.17 ±0.29	78.16 ±0.58	22.0	22.8	22.8	21.3
		FP	47.73 ±0.10	47.09 ±0.01	47.70 ±0.14	46.62 ±0.41	52.3	52.8	52.3	53.3
4	M4	SG	77.99 ±0.39	77.15 ±0.38	77.19 ±0.66	78.66 ±0.52	21.4	22.3	22.4	23.0
		FP	46.71 ±0.45	48.18 ±0.18	46.65 ±0.35	47.46 ±0.28	53.2	53.2	53.3	52.5

4.3.6 Effect of eight hours desiccation on callus weight in cotton

The effect of eight hour desiccation using silica gel and filter paper on callus culture by from two explants viz., hypocotyls and leaf bits of PKV-Rajat were studied (Table 4.7).

In case of old callus of hypocotyls, maximum weight (72.64 ± 0.27 was recorded using silica gel desiccated callus on M1 medium, whereas in new callus it was (72.15 ± 0.16) on M1 media.

In case of old callus of leaf bits, maximum weight (70.02±0.12) was observed using silica gel on M1 medium, whereas in new callus it was (71.41 ± 0.21) on M4 medium.

Maximum fresh weight after desiccation of old callus of hypocotyls using filter paper was (60.34 ± 0.09) whereas in new callus it was (60.34 ±0.52) on M2 media.

Maximum fresh weight after desiccation of old callus of leaf bits by using filter paper was (47.99 ± 0.17) and in new callus it was (47.80 ± 0.61) on medium M2.

In case of silica gel, higher desiccation percentage was recorded from new callus of hypocotyl (28.36) and in leaf bits (30.94) on M4 media, whereas in case of filter paper, highest desiccation percentage was recorded from old callus of hypocotyls (61.26) and leaf bits (60.34) on M3 and M2 media, respectively. These results are comparable to Rance (1994) who reported loss of moisture up to 60 percent of fresh weight of callus in rice.

Table 4.7 Effect of eight-hour desiccation on callus weight in cotton.

Sr. No.	Medium	Desiccators	Weight of callus after desiccation in percentage				Desiccation percent			
			Hypocotyls		Leaf bits		Hypocotyls		Leaf bits	
			Old	New	Old	New	Old	New	Old	New
1	M1	SG	72.64 ±0.27	72.08 ±0.14	70.23 ±0.1	69.75 ±0.2	27.36	28.30	29.77	28.68
		FP	41.16 ±0.30	39.38 ±0.34	39.66 ±0.1	40.37 ±0.4	58.84	58.51	60.34	59.63
2	M2	SG	71.94 ±0.10	70.86 ±0.08	69.06 ±0.21	70.57 ±0.2	28.06	27.28	28.59	30.25
		FP	40.22 ±0.21	39.83 ±0.61	40.59 ±0.2	40.38 ±0.1	59.78	58.98	59.41	59.62
3	M3	SG	69.90 ±0.29	72.15 ±0.16	72.54 ±0.2	70.02 ±0.1	27.92	27.14	29.43	30.46
		FP	38.74 ±0.22	39.41 ±0.38	40.91 ±0.4	40.91 ±0.5	61.26	59.64	59.09	59.09
4	M4	SG	70.69 ±0.1	71.70 ±0.24	71.41 ±0.2	69.1± 0.2	27.85	28.36	29.98	30.94
		FP	41.49 ±0.32	41.02 ±0.36	41.12 ±0.5	40.91 ±0.1	58.51	58.98	58.88	57.88

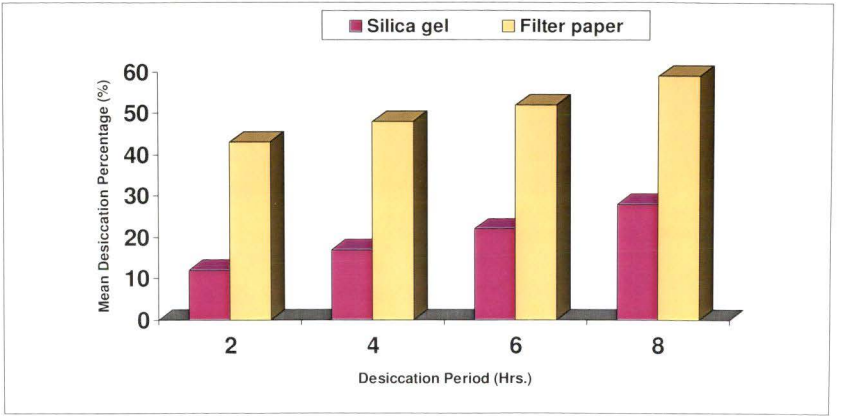


Fig. 3 Effect of desiccation treatment on callus cultures in cotton

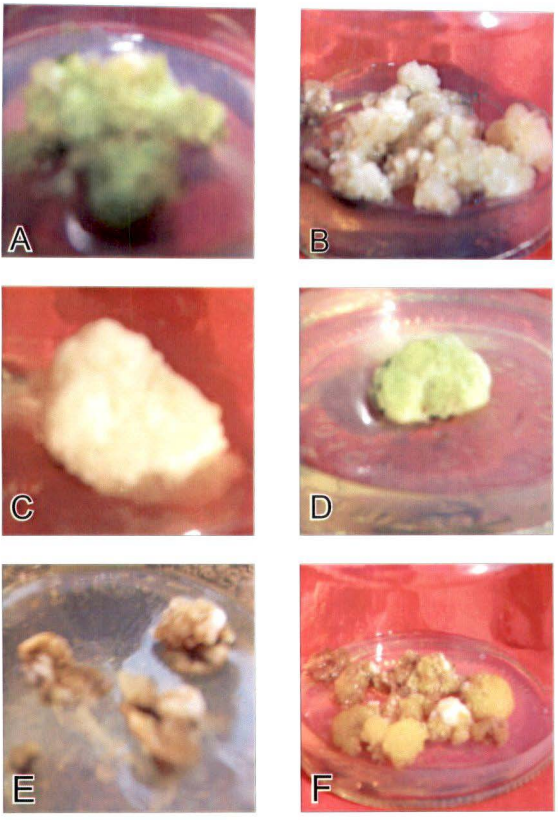


Plate 2 : Colour and texture of callus

A - Green colour callus
B - Friable watery callus
C - Compact white callus
D - Yellowish green compact callus
E - Friable brownish callus
F - Yellowish green friable callus

4.5 Effect of desiccation on regeneration responses in cotton

Effect of desiccation treatment and different media combination on somatic embryogenesis in cotton was studied 30 days after desiccation callus was transferred to the regeneration media and the data was recorded (Table 4.8).

4.5.1 Effect of desiccation on somatic embryogenesis

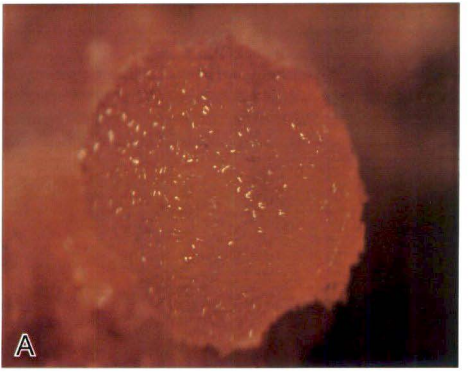
In controlled callus somatic embryo frequency was minimum as compared to desiccated callus. It was observed that 2 and 4 hrs silica gel desiccation treatment gave better response for somatic embryo development than filter paper. In 2, 4 and 6 hrs silica gel desiccation treatments, maximum number of somatic embryos were developed on SM-27, SM-36, SM-43, SM-47, SM-53, SM-59, SM-71, SM-75 and SM-84 and SM-110, respectively after 40 days of inoculation of desiccated callus on regeneration media. These results were comparable to Deng *et al.* (2009).

Filter paper desiccation treatment for 2 and 4 h showed less somatic embryos development in SM-2, SM-3, SM-5, SM-12, SM-23, SM-27, SM-42, SM-43, SM-53, SM-59, SM-60-, SM-75 media, respectively. Somatic embryogenesis was observed from non desiccated callus on media SM-2, SM-12, SM-39 SM-47, SM-53, SM-59, SM-60 and SM-84.Similar results were also observed by Smita Shingane (2008) in cotton.

Non desiccated callus showed less response for somatic embryogenesis than desiccated callus. These results were in agreement with the finding of Wagiran (2008) in Japonica rice.

4.5.2 Colour and texture of callus after desiccation

It was apparently observed that the influence of media combinations on the expression of callus colour and texture in this case was distinct and was clearly detected at the callus formation stage.



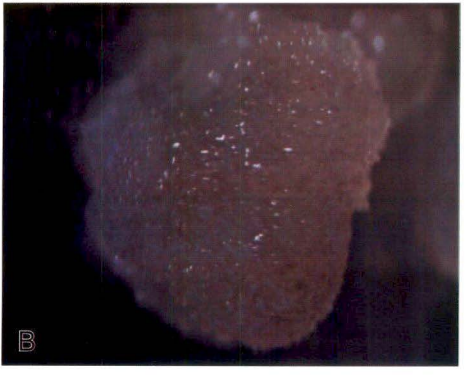


Plate 3 : Stages of somatic embryogenesis in cotton
A - Globular stage of somatic embryos
B - Heart shape stage of somatic embryos

Callus treated with filter paper desiccation treatment showed green coloured and compact white coloured callus on maximum number of media (Plate-2). Friable white callus showed maximum response to somatic embryo formation (Plate-3). Similar results were reported by Verma *et al.* (2005).Yellowish green callus, light green compact callus, friable watery callus were observed in different media treatment. The higher concentration of auxins resulted in increased friability of callus. These results are similar to the report of Trolinder and Goodin (1988) and Firozabody *et al.* (1993).

The variation with respect to colour and texture of embryogenic calli on different media combination has been observed by several authors (Finer 1988, Firozabady *et al.* 1987, Gawel *et al.* 1986, Shoemaker *et al.* 1986, Trolinder and Goodin 1988).

4.5.3 Growth Stages of somatic embryos

Globular and heart shape stages of somatic embryos were observed maximum on silica gel desiccation treatment than filter paper. (Plate 3-A and 3-B)

Desiccation also resulted in accelerated organogenesis. In some cases rhizogenesis was also observed when desiccated calli transferred to the regeneration media. Similar results were also reported by Mathusamy and Jayabalan (2000). Comparable results were also reported by Suprasanna *et al.* (2007) in sugarcane and Kumaria *et al.* (2003) in cotton.

4.5.4 Number and frequency of somatic embryos.

The maximum (13.39) percentage of globular somatic embryos were developed after 2h silica gel desiccation treatments in hypocotyls and in case of leaf bits it was (11.34) on medium SM-27 (0.75 mg/l NAA and 1.0 mg/l BAP).While heart shape stage frequency of somatic embryos was observed to be 2.99 on SM-53 (MS +0.2 mg/l Kin). Filter paper

desiccated calli beared globular stage somatic embryos up to (9.87%) in hypocotyls on medium SM-47 (2mg/l NAA+ 2mg/l BAP) and 6.67 in leaf bits on media SM-23 (0.5mg/lNAA 0.5mg/l BAP). Desiccation resulted in 12 to 20% loss of fresh weight of callus, which significantly increased the frequency of somatic embryos in *Gossypium* spp.

In non-desiccated callus, frequency of somatic embryos was minimum as compared to desiccated callus. Non desiccated calli beared globular somatic embryos up to 2.41% in hypocotyls and leaf bits1.73% on medium SM-59(MS+1mg/l BAP).Embryo initiation and maturation was also affected by media treatment. These results supported the findings of Sakhanokho *et al. (2001)* in cotton.

Several authors; Gawel *et al.* (1996), Gawel and Roback (1990), Kumar and Pental (1998), Shoemaker et al. (1986), Chand and Sahrawat (2001) have reported a low frequency of embryo maturation in cotton.

Table 4.8: Effect of desiccation treatments on somatic embryos development in cotton

Medium	Desiccation treatment	Qty of callus for SEs (mg)		Colour of callus		No of SEs		Growth of SEs		Stages of SEs		Frequency of SE	
Explants		Hy	LB	Hy	LB	Hy	LB	Hy	LB	Hy	LB	Hy	LB
1	2	3	4	5	6	7	8	9	10	11	12	13	14
SM-1	ND	183	121	YGCC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
SM-2	ND	173	138	LGCC	CWC	2	0	Good	NR	GSEM	NR	1.16	0.00
	2SG	193	109	GCC	FWB	0	12	NR	Good	GSEM	GSEM	0.00	11.01
	2FP	123	134	GCC	CWC	7	0	Good	NR	GSEM	NR	5.69	0.00
	4SG	121	142	GCC	GCC	0	0	NR	NR	NR	NR	0.00	0.00
	4FP	182	128	YGCC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
	6SG	118	112	FBW	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	6FP	137	110	YGCC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
	8S	182	132	FBW	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	8FP	140	165	FBW	CWC	0	0	NR	NR	NR	NR	0.00	0.00
SM-3	ND	199	183	FBW	FBW	0	0	NR	NR	NR	NR	0.00	0.00
	2SG	156	198	GCC	GCC	0	3	NR	Good	Rz	GSEM	0.00	1.52
	2FP	173	176	GCC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
	4SG	149	134	FBW	GCC	6	6	Good	Good	GSEM	GSEM	4.03	4.48
	4FP	152	122	LGCC	YGCC	5	0	Good	NR	GSEM	NR	3.29	0.00
	6SG	163	165	FBW	CWC	4	4	Good	Good	GSEM	GSEM	2.45	2.42
	6FP	144	143	FBW	CWC	0	0	NR	NR	NR	NR	0.00	0.00
	8SG	139	105	FW	CWC	6	3	Good	Good	GSEM	GSEM	4.32	2.86
	8FP	132	162	FW	CWC	0	0	NR	NR	NR	NR	0.00	0.00
SM-4	ND	193	192	LGCC	YGCC	0	0	NR	NR	RZ	NR	0.00	0.00
	2SG	148	138	FBW	YGCC	0	3	NR	good	NR	GSEM	0.00	2.17
	2FP	138	124	FBW	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	4SG	175	136	YGCC	LGCC	12	13	Good	Good	GSEM	GSEM	6.86	9.56
	4FP	194	173	LGCC	FW	0	0	NR	NR	Rz	Rz	0.00	0.00
	6SG	120	198	LGCC	FW	0	0	NR	Poor	NR	NR	0.00	0.00
	6FP	196	186	LGCC	CWC	0	0	NR	NR	NR	RZ	0.00	0.00
	8SG	194	156	FBW	CWC	0	0	NR	NR	NR	NR	0.00	0.00
	8FP	173	144	FBW	CWC	0	0	NR	NR	NR	NR	0.00	0.00
SM-5	ND	124	213	FBW	FBW	0	0	NR	NR	NR	NR	0.00	0.00
	2SG	129	134	LGCC	GCC	6	7	Good	Good	GSEM	GSEM	4.65	5.22
	2FP	127	164	LGCC	YGCC	3	0	Good	NR	GSEM	NR	2.36	0.00
	4SG	148	185	LGCC	YGCC	8	9	Good	Good	GSEM	GSEM	5.41	4.86
	4FP	123	209	LGCC	GCC	0	0	NR	NR	RZ	RZ	0.00	0.00
	6SG	134	126	FBW	CWC	0	0	NR	NR	RZ	RZ	0.00	0.00
	6FP	145	104	FBW	CWC	0	0	NR	NR	RZ	RZ	0.00	0.00
	8SG	173	159	LFW	GCC	0	0	NR	NR	RZ	NR	0.00	0.00
	8FP	183	98	FBW	FW	0	0	NR	NR	NR	NR	0.00	0.00
SM-6	ND	202	175	LFW	YGCC	0	0	NR	NR	NR	NR	0.00	0.00

Contd..

	2SG	184	182	FBW	GCC	6	4	Good	Good	GSEM	GSEM	3.26	2.20
	2FP	183	137	LGCC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
	4SG	183	133	GCC	GCC	0	0	NR	NR	RZ	RZ	0.00	0.00
	4FP	184	148	YGCC	YGCC	5	6	Good	Good	GSEM	GSEM	2.72	4.05
	6SG	147	129	YGCC	FBW	0	0	NR	NR	NR	NR	0.00	0.00
	6FP	193	192	LGCC	YGCC	0	0	NR	NR	RZ	NR	0.00	0.00
	8SG	193	176	FBW	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	8FP	282	163	FBW	FBW	0	0	NR	NR	NR	NR	0.00	0.00
SM-7	ND	172	115	LGCC	LGCC	0	0	NR	NR	NR	NR	0.00	0.00
	2SG	190	154	YGCC	GCC	4	3	Poor	Good	GSEM	GSEM	2.11	1.95
	2FP	128	186	YGCC	YGCC	NR	NR	NR	NR	NR	NR	0.00	0.00
	4SG	183	123	LGCC	YGCC	11	7	Poor	Good	GSEM	GSEM	6.01	5.69
	4FP	197	147	YGCC	YGCC	0	0	NR	NR	RZ	NR	0.00	0.00
	6SG	172	135	GCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	6FP	183	177	YGCC	CWC	0	0	NR	NR	RZ	RZ	0.00	0.00
	8SG	199	211	YGCC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
	8FP	174	172	YGCC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
SM-8	ND	121	142	GCC	GCC	0	0	NR	NR	NR	NR	0.00	0.00
	2SG	185	137	LGCC	YGCC	3	8	Good	Good	GSEM	GSEM	1.62	5.84
	2FP	124	152	LGCC	GCC	0	0	NR	NR	NR	NR	0.00	0.00
	4SG	136	141	YGCC	GCC	0	0	NR	NR	NR	NR	0.00	0.00
	4FP	123	179	GCC	GCC	0	0	NR	NR	NR	NR	0.00	0.00
	6SG	125	167	YGCC	FBW	0	0	NR	NR	NR	NR	0.00	0.00
	6FP	152	155	LGCC	FW	0	0	NR	NR	NR	NR	0.00	0.00
	8SG	143	157	FBW	FBW	9	10	Good	Good	GSEM	GSEM	6.29	6.37
	8FP	152	138	FBW	CWC	0	0	NR	NR	RZ	NR	0.00	0.00
SM-9	ND	219	182	CWC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
	2SG	140	126	LFW	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	2FP	181	184	FBW	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	4SG	180	172	FBW	FBW	0	0	NR	NR	RZ	RZ	0.00	0.00
	4FP	199	160	GCC	FBW	0	0	NR	NR	NR	NR	0.00	0.00
	6SG	187	168	FBW	FBW	0	0	NR	NR	NR	NR	0.00	0.00
	6FP	182	148	LFW	LGCC	0	0	NR	NR	NR	NR	0.00	0.00
	8SG	169	125	FBW	FW	0	0	NR	NR	NR	NR	0.00	0.00
	8FP	166	153	FBW	FW	0	0	NR	NR	NR	NR	0.00	0.00
SM-10	ND	124	213	FBW	FBW	0	0	NR	NR	NR	NR	0.00	0.00
	2SG	168	172	FBW	LGCC	3	2	Good	Good	GSEM	GSEM	1.79	1.16
	2FP	191	185	FBW	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	4SG	109	164	LGCC	YGCC	12	7	Good	Good	GSEM	GSEM	11.01	4.27
	4FP	119	140	LGCC	YGCC	NR	0	NR	NR	NR	NR	0.00	0.00
	6SG	204	131	LGCC	YGCC	9	0	Good	NR	GSEM	NR	4.41	0.00
	6FP	197	173	YGCC	GCC	0	0	NR	NR	NR	NR	0.00	0.00
	8SG	97	168	FBW	FBW	0	0	NR	NR	NR	NR	0.00	0.00
	8FP	207	209	FBW	FBW	0	0	NR	NR	NR	NR	0.00	0.00
SM-11	ND	199	161	FW	FW	0	0	NR	NR	NR	NR	0.00	0.00
	2SG	211	116	YGCC	FBW	10	3	Good	Good	GSEM	GSEM	4.74	2.59

Contd..

	2FP	193	212	YGCC	FBW	0	0	NR	NR	NR	NR	0.00	0.00
	4SG	126	169	YGCC	FBW	9	5	Very good	Good	GSEM	GSEM	7.14	2.96
	4FP	196	145	YGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	6SG	189	103	LGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	6FP	172	115	LGCC	LGCC	0	0	NR	NR	NR	NR	0.00	0.00
	8SG	163	127	FBW	LWF	8	6	Poor	Good	GSEM	GSEM	4.91	4.72
	8FP	155	147	FBW	FW	0	0	NR	NR	NR	NR	0.00	0.00
SM-12	ND	184	135	CWC	CWC	3	0	Good	NR	GSEM	NR	1.63	0.00
	2SG	163	183	YGCC	GCC	13	10	Good	Good	GSEM	GSEM	7.98	5.46
	2FP	182	190	LGCC	LGCC	7	4	Good	Good	GSEM	GSEM	3.85	2.11
	4SG	180	149	GCC	LGCC	9	7	Good	Good	GSEM	GSEM	5.00	4.70
	4FP	178	182	GCC	LGCC	0	0	NR	NR	Rz	NR	0.00	0.00
	6SG	190	164	GCC	YGCC	0	0	NR	NR	Rz	NR	0.00	0.00
	6FP	195	175	YGCC	FBW	0	0	NR	Good	Rz	GSEM	0.00	0.00
	8SG	173	231	FBW	FW	6	4	Good	Good	GSEM	GSEM	3.47	1.73
	8FP	177	273	LFW	FBW	0	0	NR	NR	NR	NR	0.00	0.00
SM-13	ND	173	138	LGCC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
	2SG	166	195	FBW	GCC	0	0	NR	NR	NR	NR	0.00	0.00
	2FP	172	188	FBW	GCC	0	0	NR	NR	NR	NR	0.00	0.00
	4SG	192	193	YGCC	GCC	0	0	NR	NR	NR	NR	0.00	0.00
	4FP	205	183	GCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	6SG	202	175	LFW	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	6FP	183	237	CWC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	8SG	146	254	CWC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	8FP	122	28	FBW	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
SM-14	ND	193	121	LGCC	LGCC	0	0	NR	NR	NR	NR	0.00	0.00
	2SG	223	191	LFW	LFW	0	0	NR	NR	NR	NR	0.00	0.00
	2FP	193	173	YGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	4SG	203	253	GCC	GCC	0	0	NR	NR	NR	NR	0.00	0.00
	4FP	195	184	GCC	GCC	0	0	NR	NR	NR	NR	0.00	0.00
	6SG	175	174	GCC	GCC	0	0	NR	NR	NR	NR	0.00	0.00
	6FP	254	168	GCC	GCC	0	0	NR	NR	NR	NR	0.00	0.00
	8SG	243	123	YGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	8FP	274	173	LGCC	LGCC	0	0	NR	NR	Rz	NR	0.00	0.00
SM-15	ND	131	136	FW	FW	0	0	NR	NR	NR	NR	0.00	0.00
	2SG	185	182	FBW	FBW	7	4	Good	Good	GSEM	GSEM	3.78	2.20
	2FP	199	161	FW	FW	0	0	NR	NR	NR	NR	0.00	0.00
	4SG	221	107	FBW	FBW	0	0	NR	NR	GSEM	NR	0.00	0.00
	4FP	221	243	FBW	FBW	0	0	NR	NR	NR	NR	0.00	0.00
	6SG	264	264	FBW	FBW	0	0	NR	NR	NR	NR	0.00	0.00
	6FP	283	180	YGCC	YGCC	0	0	NR	NR	Rz	NR	0.00	0.00
	8SG	194	189	YGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	8FP	134	172	LGCC	LGCC	0	0	NR	NR	NR	NR	0.00	0.00
SM-16	ND	173	GCC	GCC	GCC	0	0	NR	NR	NR	NR	0.00	0.00
	2SG	174	166	GCC	GCC	13	13	Good	Good	GSEM	GSEM	7.47	7.83
	2FP	173	47	GCC	GCC	0	0	NR	NR	NR	NR	0.00	0.00

Contd..

	4FP	158	146	YGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	6SG	83	133	YGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	6FP	273	102	GCC	GCC	0	0	NR	NR	NR	NR	0.00	0.00
	8SG	224	118	LGCC	LGCC	0	0	NR	NR	NR	NR	0.00	0.00
	8FP	186	127	LGCC	LGCC	0	0	NR	NR	NR	NR	0.00	0.00
SM-21	ND	204	187	LGCC	LGCC	0	0	NR	NR	NR	NR	0.00	0.00
	2SG	188	145	LGCC	LGCC	0	0	NR	NR	NR	NR	0.00	0.00
	2FP	153	132	YGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	4SG	151	184	LGCC	LGCC	0	0	NR	NR	NR	NR	0.00	0.00

Contd..

	4FP	170	235	LGCC	LGCC	0	0	NR	NR	NR	NR	0.00	0.00
	6SG	164	137	LGCC	LGCC	0	0	NR	NR	NR	NR	0.00	0.00
	6FP	179	121	LGCC	LGCC	0	0	NR	NR	NR	NR	0.00	0.00
	8SG	184	172	YGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	8FP	201	204	CWC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
SM-22	ND	182	194	YGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	2SG	236	114	GCC	GCC	0	0	NR	NR	NR	NR	0.00	0.00
	2FP	223	173	GCC	GCC	0	0	NR	NR	NR	NR	0.00	0.00
	4SG	172	183	GCC	GCC	0	0	NR	NR	NR	NR	0.00	0.00
	4FP	184	193	GCC	GCC	0	0	NR	NR	NR	NR	0.00	0.00
	6SG	284	124	GCC	GCC	0	0	NR	NR	NR	NR	0.00	0.00
	6FP	179	127	YGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	8SG	230	113	YGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	8FP	233	194	YGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
SM-23	ND	183	164	GCC	GCC	0	0	NR	NR	NR	NR	0.00	0.00
	2SG	216	191	GCC	GCC	0	0	NR	NR	NR	NR	0.00	0.00
	2FP	184	187	GCC	GCC	0	0	NR	NR	NR	NR	0.00	0.00
	4SG	144	183	GCC	GCC	10	10	Good	Good	GSEM	GSEM	6.94	5.46
	4FP	162	165	GCC	GCC	11	11	Good	Good	GSEM	GSEM	6.79	6.67
	6SG	173	13	GCC	GCC	0	0	NR	NR	NR	NR	0.00	0.00
	6FP	184	183	GCC	GCC	0	0	NR	NR	NR	NR	0.00	0.00
	8SG	195	194	GCC	GCC	0	0	NR	NR	NR	NR	0.00	0.00
	8FP	195	137	GCC	GCC	0	0	NR	NR	Rz	Rz	0.00	0.00
SM-24	ND	194	184	FW	FW	0	0	NR	NR	NR	NR	0.00	0.00
	2SG	248	213	YGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	2FP	127	193	YGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	4SG	234	93	YGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	4FP	173	192	YGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	6SG	172	173	CWC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
	6FP	234	182	CWC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
	8SG	126	163	CWC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
	8FP	272	166	CWC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
SM-25	ND	121	211	LGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	2SG	284	154	LGCC	LGCC	0	0	NR	NR	NR	NR	0.00	0.00
	2FP	193	168	LGCC	LGCC	0	0	NR	NR	NR	NR	0.00	0.00
	4SG	204	187	LGCC	LGCC	0	0	NR	NR	NR	NR	0.00	0.00
	4FP	193	121	LGCC	LGCC	0	0	NR	NR	NR	NR	0.00	0.00
	6SG	185	209	FBW	FBW	0	0	NR	NR	NR	NR	0.00	0.00
	6FP	149	211	FBW	FBW	0	0	NR	NR	NR	NR	0.00	0.00
	8SG	137	217	FBW	FBW	0	0	NR	NR	NR	NR	0.00	0.00
	8FP	133	183	FBW	FBW	0	0	NR	NR	NR	NR	0.00	0.00
SM-26	ND	220	173	GCC	GCC	0	0	NR	NR	NR	NR	0.00	0.00
	2SG	170	224	FBW	FBW	0	0	NR	NR	NR	NR	0.00	0.00
	2FP	172	93	FBW	FBW	0	0	NR	NR	NR	NR	0.00	0.00
	4SG	140	190	FBW	FBW	0	0	NR	NR	NR	NR	0.00	0.00
	4FP	138	92	FBW	FBW	0	0	NR	NR	NR	NR	0.00	0.00

Contd..

	6SG	131	124	FBW	FBW	0	0	NR	NR	NR	NR	0.00	0.00
	6FP	183	225	FW	FW	0	0	NR	NR	NR	NR	0.00	0.00
	8SG	184	184	FBW	FBW	0	0	NR	NR	NR	NR	0.00	0.00
	8FP	194	190	FBW	FBW	1	5	NR	NR	NR	NR	0.52	2.63
SM-27	ND	187	182	FBW	FBW	3	0	Good	NR	GSEM	NR	1.60	0.00
	2SG	120	218	YGCC	YGCC	13	8	Good	Good	GSEM	GSEM	10.83	3.67
	2FP	112	97	YGCC	YGCC	15	11	Good	Good	GSEM	GSEM	13.39	11.34
	4SG	220	165	YGCC	YGCC	9	10	Good	Good	GSEM	GSEM	4.09	6.06
	4FP	211	183	YGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	6SG	212	194	YGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	6FP	220	199	YGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	8SG	162	219	LGCC	LGCC	0	0	NR	NR	NR	NR	0.00	0.00
	8FP	173	172	LGCC	LGCC	0	0	NR	NR	NR	NR	0.00	0.00
SM-28	ND	121	211	LGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	2SG	212	211	FBW	FBW	0	0	NR	NR	NR	NR	0.00	0.00
	2FP	78	234	FBW	FBW	0	0	NR	NR	NR	NR	0.00	0.00
	4SG	94	201	FBW	FBW	0	0	NR	NR	NR	NR	0.00	0.00
	4FP	183	148	FBW	FBW	0	0	NR	NR	NR	NR	0.00	0.00
	6SG	193	183	FBW	FBW	0	0	NR	NR	NR	NR	0.00	0.00
	6FP	243	191	FBW	FBW	0	0	NR	NR	NR	NR	0.00	0.00
	8SG	274	193	FBW	FBW	0	0	NR	NR	NR	NR	0.00	0.00
	8FP	185	142	FBW	FBW	0	0	NR	NR	NR	NR	0.00	0.00
SM-29	ND	121	211	LGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	2SG	199	183	CWC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
	2FP	221	192	CWC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
	4SG	221	184	CWC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
	4FP	264	194	CWC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
	6SG	283	175	CWC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
	6FP	194	173	CWC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
	8SG	134	154	CWC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
	8FP	174	166	CWC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
SM-30	ND	184	166	GCC	FBW	0	0	NR	NR	NR	NR	0.00	0.00
	2SG	173	89	CWC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
	2FP	194	237	CWC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
	4SG	148	221	CWC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
	4FP	284	181	CWC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
	6SG	194	203	CWC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
	6FP	175	112	CWC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
	8SG	174	125	CWC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
	8FP	193	201	CWC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
SM-31	ND	194	184	FW	FW	0	0	NR	NR	NR	NR	0.00	0.00
	2SG	173	225	CWC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
	2FP	137	193	FW	FW	0	0	NR	NR	NR	NR	0.00	0.00
	4SG	133	139	FW	FW	0	0	NR	NR	NR	NR	0.00	0.00
	4FP	123	147	FW	FW	0	0	NR	NR	NR	NR	0.00	0.00
	6SG	175	220	FW	FW	0	0	NR	NR	NR	NR	0.00	0.00

Contd..

	6FP	193	183	FW	FW	0	0	NR	NR	NR	NR	0.00	0.00
	8SG	239	183	FW	FW	0	0	NR	NR	NR	NR	0.00	0.00
	8FP	184	219	CWC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
SM-32	ND	183	164	GCC	GCC	0	0	NR	NR	NR	NR	0.00	0.00
	2SG	128	134	GCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	2FP	172	173	GCC	FW	0	0	NR	NR	NR	NR	0.00	0.00
	4SG	184	174	GCC	FBW	0	0	NR	NR	NR	NR	0.00	0.00
	4FP	198	163	GCC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
	6SG	138	184	YGCC	FBW	0	0	NR	NR	NR	NR	0.00	0.00
	6FP	146	115	YGCC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
	8SG	133	135	YGCC	FW	0	0	NR	NR	NR	NR	0.00	0.00
	8FP	102	145	YGCC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
SM-33	ND	193	176	CWC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
	2SG	118	128	GCC	GCC	3	2	Good	Good	GSEM	GSEM	2.54	1.56
	2FP	127	165	CWC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
	4SG	145	183	CWC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	4FP	132	121	CWC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
	6SG	184	109	YGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	6FP	235	134	CWC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
	8SG	137	142	YGCC	FW	0	0	NR	NR	NR	NR	0.00	0.00
	8FP	121	128	CWC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
SM-34	ND	212	194	YGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	2SG	172	112	YGCC	GCC	4	6	Poor	Good	GSEM	GSEM	2.33	5.36
	2FP	204	110	FWB	FWB	0	0	NR	NR	NR	NR	0.00	0.00
	4SG	114	132	YGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	4FP	173	165	FW	WCC	0	0	NR	NR	NR	NR	0.00	0.00
	6SG	183	198	YGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	6FP	193	176	CWC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
	8SG	124	134	FWB	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	8FP	127	122	CWC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
SM-35	ND	109	225	CWC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
	2SG	113	165	GCC	FBW	7	0	Good	NR	GSEM	NR	6.19	0.00
	2FP	194	143	FBW	FBW	0	0	NR	NR	NR	NR	0.00	0.00
	4SG	191	105	GCC	FBW	0	0	Good	NR	GSEM	NR	0.00	0.00
	4FP	187	162	FW	FBW	0	0	NR	NR	NR	NR	0.00	0.00
	6SG	183	138	FBW	FBW	0	0	NR	NR	NR	NR	0.00	0.00
	6FP	165	124	CWC	FBW	0	0	NR	NR	NR	NR	0.00	0.00
	8S	131	136	FW	FW	7	0	Good	NR	GSEM	NR	5.34	0.00
	8FP	183	173	FBW	FBW	0	0	NR	NR	NR	NR	0.00	0.00
SM-36	ND	191	138	LGCC	LGCC	4	0	NR	Good	NR	GSEM	2.09	0.00
	2SG	194	198	FBW	FBW	10	8	Good	Good	GSEM	GSEM	5.18	5.56
	2FP	137	186	YGCC	YGCC	10	9	Good	Good	GSEM	GSEM	7.30	4.84
	4SG	213	156	YGCC	YGCC	12	8	Very good	Good	GSEM	GSEM	5.63	5.13
	4FP	193	144	YGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	6SG	93	134	YGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	6FP	192	164	YGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00

Contd..

	8SG	173	185	YGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	8FP	182	209	LGCC	LGCC	0	0	NR	NR	NR	NR	0.00	0.00
SM-37	ND	162	224	LGCC	LGCC	0	0	NR	NR	NR	NR	0.00	0.00
	2SG	163	126	LGCC	LGCC	0	0	NR	NR	NR	NR	0.00	0.00
	2FP	166	104	FW	FBW	0	0	NR	NR	NR	NR	0.00	0.00
	4SG	154	159	FBW	FBW	0	0	NR	NR	NR	NR	0.00	0.00
	4FP	168	98	FBW	FBW	0	0	NR	NR	NR	NR	0.00	0.00
	6SG	187	182	FBW	FBW	3	0	Good	NR	GSEM	NR	1.60	0.00
	6FP	121	137	FBW	FBW	0	0	NR	NR	NR	NR	0.00	0.00
	8SJ	209	133	FBW	FBW	0	0	NR	NR	NR	NR	0.00	0.00
	8FP	211	148	FBW	FBW	0	0	NR	NR	NR	NR	0.00	0.00
SM-38	ND	121	142	GCC	GCC	0	0	NR	NR	NR	NR	0.00	0.00
	2SG	217	129	FBW	FBW	0	0	NR	NR	NR	NR	0.00	0.00
	2FP	183	192	CWC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
	4SG	224	176	CWC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
	4FP	93	163	CWC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
	6SG	190	154	CWC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
	6FP	92	186	CWC	CWC	0	0	NR	NR	RZ	NR	0.00	0.00
	8SG	124	123	CWC	CWC	0	0	NR	NR	RZ	NR	0.00	0.00
	8FP	225	147	CWC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
SM-39	ND	118	128	GCC	GCC	3	0	Good	NR	GSEM	NR	1.63	0.00
	2SG	184	135	GCC	GCC	3	2	Good	Good	GSEM	GSEM	2.54	1.56
	2FP	190	177	CWC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
	4SG	218	211	CWC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
	4FP	97	172	CWC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
	6SG	165	137	CWC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
	6FP	183	152	CWC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
	8SG	194	141	CWC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
	8FP	199	179	CWC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
SM-40	ND	199	183	FBW	FBW	0	0	NR	NR	NR	NR	0.00	0.00
	2SG	219	167	CWC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
	2FP	172	155	CWC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
	4SG	211	157	FW	FW	0	0	NR	NR	NR	NR	0.00	0.00
	4FP	234	138	FW	FW	0	0	NR	NR	NR	NR	0.00	0.00
	6SG	201	126	FW	FW	0	0	NR	NR	NR	NR	0.00	0.00
	6FP	148	184	FW	FW	0	0	NR	NR	NR	NR	0.00	0.00
	8SG	183	172	FW	FW	0	0	NR	NR	NR	NR	0.00	0.00
	8FP	191	160	FW	FW	0	0	NR	NR	NR	NR	0.00	0.00
SM-41	ND	137	209	LGCC	LGCC	0	0	NR	NR	NR	NR	0.00	0.00
	2SG	193	168	CWC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
	2FP	142	148	YGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	4SG	183	125	FW	FW	0	0	NR	NR	NR	NR	0.00	0.00
	4FP	192	153	FBW	FBW	0	0	NR	NR	NR	NR	0.00	0.00
	6SG	184	172	CWC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
	6FP	194	185	FBW	FBW	0	0	NR	NR	NR	NR	0.00	0.00
	8SG	175	164	CWC	CWC	0	0	NR	NR	NR	NR	0.00	0.00

Contd..

	8FP	173	140	FW	FW	0	0	NR	NR	NR	NR	0.00	0.00
SM-42	ND	162	224	LGCC	LGCC	0	0	NR	NR	NR	NR	0.00	0.00
	2SG	154	131	GCC	GCC	4	0	Good	NR	GSEM	NR	2.60	0.00
	2FP	166	173	GCC	GCC	4	3	Good	Good	GSEM	GSEM	2.41	1.73
	4SG	89	168	CWC	CWC	0	0	NR	NR	Rz	Rz	0.00	0.00
	4FP	237	209	FWB	FWB	0	0	NN	NR	Rz	Rz	0.00	0.00
	6SG	221	116	GCC	GCC	6	5	Good	Good	GSEM	GSEM	2.71	4.31
	6FP	181	212	CWC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
	8SG	203	169	YGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	8FP	112	145	CWC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
SM-43	ND	183	164	FBW	FBW	3	3	Good	Good	GSEM	GSEM	1.64	1.83
	2SG	125	103	GCC	GCC	10	11	Good	Good	GSEM	GSEM	8.00	10.68
	2FP	201	115	YGCC	YGCC	9	8	Good	Good	GSEM	GSEM	4.48	6.96
	4SG	225	127	GCC	GCC	9	9	Good	Good	GSEM	GSEM	4.00	7.09
	4FP	193	147	CWC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
	6SG	139	183	GCC	GCC	6	6	Good	NR	GSEM	NR	4.32	3.28
	6FP	147	190	CWC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
	8SG	220	149	CWC	CWC	0	0	NR	NR	Rz	NR	0.00	0.00
	8FP	183	182	CWC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
SM-44	ND	131	136	FW	FW	0	0	NR	NR	NR	NR	0.00	0.00
	2SG	183	164	GCC	GCC	3	3	Good	Good	GSEM	GSEM	1.64	1.83
	2FP	219	175	CWC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
	4SG	134	231	YGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	4FP	173	273	CWC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
	6SG	174	195	YGCC	YGCC	0	0	NR	NR	Rz	NR	0.00	0.00
	6FP	163	188	CWC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
	8SG	184	193	FW	FW	0	0	NR	NR	NR	NR	0.00	0.00
	8FP	115	183	CWC	CWC	0	0	NR	NR	Rz	NR	0.00	0.00
SM-45	ND	183	164	FBW	FBW	0	0	NR	NR	NR	NR	0.00	0.00
	2SG	135	175	GCC	GCC	7	6	Good	Good	GSEM	GSEM	5.19	3.43
	2FP	145	237	FWB	FWB	0	0	NR	NR	NR	NR	0.00	0.00
	4SG	128	254	YGCC	YGCC	5	0	Good	NR	GSEM	NR	3.91	0.00
	4FP	165	28	CWC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
	6SG	183	191	YGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	6FP	121	173	CWC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
	8SG	109	253	YGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	8FP	134	184	CWC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
SM-46	ND	199	183	FBW	FBW	0	0	NR	NR	NR	NR	0.00	0.00
	2SG	142	174	FBW	FBW	8	0	Good	NR	GSEM	NR	5.63	0.00
	2FP	128	168	FBW	FBW	5	0	NR	NR	NR	NR	3.91	0.00
	4SG	112	123	FBW	FBW	0	0	NR	NR	NR	NR	0.00	0.00
	4FP	110	173	FBW	FBW	0	0	NR	NR	NR	NR	0.00	0.00
	6SG	132	182	FBW	FBW	0	0	NR	NR	NR	NR	0.00	0.00
	6FP	165	161	FBW	FBW	0	0	NR	NR	NR	NR	0.00	0.00
	8SG	198	107	FW	FW	0	0	NR	NR	NR	NR	0.00	0.00
	8FP	176	243	FBW	FBW	0	0	NR	NR	NR	NR	0.00	0.00

Contd..

SM-47	ND	122	209	YGCC	YGCC	3	0	Good	NR	GSEM	NR	1.63	0.00
	2SG	134	264	FBW	FBW	10	10	Good	Good	GSEM	GSEM	6.99	5.81
	2FP	122	180	YGCC	YGCC	12	9	Good	Good	GSEM	GSEM	9.84	5.00
	4SG	165	189	YGCC	YGCC	15	12	Very good	Good	GSEM	GSEM	9.09	6.35
	4FP	143	172	YGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	6SG	105	166	YGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	6FP	162	47	YGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	8SG	138	143	YGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	8FP	124	175	LGCC	LGCC	0	0	NR	NR	NR	NR	0.00	0.00
SM-48	ND	121	211	LGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	2SG	136	183	GCC	LGCC	9	9	Good	NR	GSEM	NR	6.62	4.92
	2FP	173	108	FBW	FBW	0	0	NR	NR	NR	NR	0.00	0.00
	4SG	198	165	YGCC	FBW	9	0	Good	NR	GSEM	NR	4.55	0.00
	4FP	186	172	FBW	FBW	0	0	NR	NR	NR	NR	0.00	0.00
	6SG	156	184	FBW	FBW	0	0	NR	NR	NR	NR	0.00	0.00
	6FP	144	197	FBW	FBW	0	0	NR	NR	NR	NR	0.00	0.00
	8SG	183	195	FBW	FBW	0	0	NR	NR	NR	NR	0.00	0.00
	8FP	183	164	FBW	FBW	0	0	NR	NR	NR	NR	0.00	0.00
SM-49	ND	124	213	FBW	FBW	0	0	NR	NR	NR	NR	0.00	0.00
	2SG	219	181	GCC	FBW	8	0	Good	NR	GSEM	NR	3.65	0.00
	2FP	134	178	CWC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
	4SG	173	193	CWC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
	4FP	174	182	CWC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
	6SG	163	184	CWC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
	6FP	184	154	CWC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
	8SG	115	171	CWC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
	8FP	135	149	CWC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
SM-50	ND	109	225	CWC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
	2SG	145	236	CWC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
	2FP	128	199	CWC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
	4SG	165	176	CWC	CWC	4	0	Good	NR	GSEM	NR	2.42	0.00
	4FP	183	196	CWC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
	6SG	148	188	CWC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
	6FP	183	192	CWC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
	8SG	191	182	CWC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
	8FP	193	120	CWC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
SM-51	ND	220	173	GCC	GCC	0	0	NR	NR	NR	NR	0.00	0.00
	2SG	142	111	GCC	WCC	5	0	Good	NR	GSEM	NR	3.52	0.00
	2FP	183	148	CWC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
	4SG	192	128	FW	FW	0	0	NR	NR	NR	NR	0.00	0.00
	4FP	184	172	FW	FW	0	0	NR	NR	NR	NR	0.00	0.00
	6SG	194	184	FW	FW	0	0	NR	NR	NR	NR	0.00	0.00
	6FP	175	198	FW	FW	0	0	NR	NR	NR	NR	0.00	0.00
	8SG	173	138	FW	FW	0	0	NR	NR	NR	NR	0.00	0.00
	8FP	154	146	FW	FW	0	0	NR	NR	NR	NR	0.00	0.00
SM-52	ND	217	129	FBW	FBW	0	0	NR	NR	NR	NR	0.00	0.00

Contd..

	2SG	166	133	YGCC	WCC	0	0	NR	NR	NR	NR	0.00	0.00
	2FP	89	102	YGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	4SG	237	118	FW	FW	0	0	NR	NR	NR	NR	0.00	0.00
	4FP	221	127	FW	FBW	0	0	NR	NR	NR	NR	0.00	0.00
	6SG	181	145	FBW	WCC	0	0	NR	NR	NR	NR	0.00	0.00
	6FP	203	132	CWC	FBW	0	0	NR	NR	NR	NR	0.00	0.00
	8SG	112	184	CWC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
	8FP	125	235	FW	FW	0	0	NR	NR	NR	NR	0.00	0.00
SM-53	ND	184	135	CWC	CWC	3	0	Good	NR	GSEM	NR	1.63	0.00
	2SG	201	137	GCC	GCC	6	4	Very good	Very good	HSSEM	HSSEM	2.99	2.92
	2FP	225	121	YGCC	YGCC	6	4	Good	Good	GSEM	GSEM	2.67	3.31
	4SG	193	172	GCC	GCC	7	3	Good	Good	GSEM	GSEM	3.63	1.74
	4FP	139	204	YGCC	YGCC	0	0	NR	NR	Rz	NR	0.00	0.00
	6SG	147	114	GCC	GCC	0	0	NR	NR	NR	NR	0.00	0.00
	6FP	220	173	GCC	GCC	0	0	NR	NR	NR	NR	0.00	0.00
	8SG	183	183	GCC	GCC	0	0	NR	NR	NR	NR	0.00	0.00
	8FP	183	193	YGCC	YGCC	0	0	NR	NR	Rz	NR	0.00	0.00
SM-54	ND	273	102	GCC	GCC	0	0	NR	NR	NR	NR	0.00	0.00
	2SG	219	124	LFW	LFW	0	0	NR	NR	Rz	NR	0.00	0.00
	2FP	134	127	YGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	4SG	173	113	YGCC	YGCC	10	0	Good	NR	GSEM	NR	5.78	0.00
	4FP	174	194	LGCC	LGCC	0	0	NR	NR	NR	NR	0.00	0.00
	6SG	163	191	FBW	FBW	0	0	NR	NR	NR	NR	0.00	0.00
	6FP	184	187	CWC	FBW	0	0	NR	NR	NR	NR	0.00	0.00
	8SG	115	183	FBW	FBW	0	0	NR	NR	NR	NR	0.00	0.00
	8FP	135	165	CWC	LFW	0	0	NR	NR	NR	NR	0.00	0.00
SM-55	ND	162	224	LGCC	LGCC	0	0	NR	NR	NR	NR	0.00	0.00
	2SG	145	13	GCC	GCC	0	0	NR	NR	NR	NR	0.00	0.00
	2FP	128	183	YGCC	YGCC	0	0	NR	NR	Rz	Rz	0.00	0.00
	4SG	165	194	YGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	4FP	183	137	LGCC	LGCC	0	0	NR	NR	NR	NR	0.00	0.00
	6SG	121	213	LGCC	LGCC	0	0	NR	NR	NR	NR	0.00	0.00
	6FP	109	193	FBW	FBW	0	0	NR	NR	NR	NR	0.00	0.00
	8SG	134	93	FW	FW	0	0	NR	NR	NR	NR	0.00	0.00
	8FP	142	192	CWC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
SM-56	ND	144	194	YGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	2SG	128	173	CWC	CWC	4	2	Good	NR	GSEM	NR	3.13	1.16
	2FP	112	182	LGCC	LGCC	0	0	NR	NR	NR	NR	0.00	0.00
	4SG	110	163	FBW	FBW	0	0	NR	NR	NR	NR	0.00	0.00
	4FP	132	166	FW	FW	0	0	NR	NR	NR	NR	0.00	0.00
	6SG	165	154	YGCC	FBW	7	0	Good	NR	GSEM	NR	4.24	0.00
	6FP	198	168	CWC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
	8SG	176	187	LGCC	LGCC	0	0	NR	NR	NR	NR	0.00	0.00
	8FP	134	121	YGCC	YGCC	0	0	NR	NR	Rz	NR	0.00	0.00
SM-57	ND	121	142	GCC	GCC	0	0	NR	NR	NR	NR	0.00	0.00
	2SG	122	209	YGCC	YGCC	4	0	Good	NR	GSEM	NR	3.28	0.00

Contd..

	2FP	165	211	YGCC	YGCC	0	0	NR	NR	Rz	NR	0.00	0.00
	4SG	143	217	YGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	4FP	105	183	GCC	GCC	8	0	Good	NR	GSEM	NR	7.62	0.00
	6SG	162	224	LGCC	LGCC	0	0	NR	NR	NR	NR	0.00	0.00
	6FP	138	93	LGCC	LGCC	0	0	NR	NR	NR	NR	0.00	0.00
	8SG	124	190	LGCC	LGCC	0	0	NR	NR	NR	NR	0.00	0.00
	8FP	136	92	YGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
SM-58	ND	165	225	YGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	2SG	173	124	LGCC	LGCC	0	0	NR	NR	NR	NR	0.00	0.00
	2FP	198	225	LGCC	LGCC	0	0	NR	NR	NR	NR	0.00	0.00
	4SG	186	184	LGCC	LGCC	0	0	NR	NR	NR	NR	0.00	0.00
	4FP	156	190	LGCC	LGCC	0	0	NR	NR	NR	NR	0.00	0.00
	6SG	172	218	YGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	6FP	133	97	CWC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
	8SG	109	165	GCC	GCC	0	0	NR	NR	NR	NR	0.00	0.00
	8FP	118	183	GCC	GCC	0	0	NR	NR	NR	NR	0.00	0.00
SM-59	ND	166	173	GCC	GCC	4	3	Good	Good	GSEM	GSEM	2.41	1.73
	2SG	123	194	GCC	GCC	12	0	Good	NR	GSEM	NR	9.76	0.00
	2FP	183	199	GCC	GCC	10	0	Good	NR	GSEM	NR	5.46	0.00
	4SG	193	219	GCC	GCC	11	0	Good	NR	GSEM	NR	5.70	0.00
	4FP	123	172	YGCC	YGCC	0	0	NR	NR	Rz	NR	0.00	0.00
	6SG	121	211	LGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	6FP	182	234	YGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	8SG	118	201	GCC	GCC	6	0	Good	NR	GSEM	NR	5.08	0.00
	8FP	137	148	GCC	GCC	0	0	NR	NR	Rz	NR	0.00	0.00
SM-60	ND	184	135	CWC	CWC	3	0	Good	NR	GSEM	NR	1.63	0.00
	2SG	182	183	GCC	GCC	8	5	Good	Good	GSEM	GSEM	4.40	2.73
	2FP	140	191	GCC	GCC	8	7	Good	Good	GSEM	GSEM	5.71	3.66
	4SG	156	193	GCC	GCC	0	0	NR	NR	NR	NR	0.00	0.00
	4FP	173	142	GCC	GCC	0	0	NR	NR	NR	NR	0.00	0.00
	6SG	149	183	GCC	GCC	0	0	NR	NR	NR	NR	0.00	0.00
	6FP	152	192	GCC	GCC	0	0	NR	NR	Rz	Rz	0.00	0.00
	8SG	163	184	YGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	8FP	144	194	YGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
SM-61	ND	183	183	FBW	FBW	0	0	NR	NR	NR	NR	0.00	0.00
	2SG	139	175	YGCC	YGCC	0	0	NR	NR	Rz	NR	0.00	0.00
	2FP	132	173	LGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	4SG	148	154	CWC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
	4FP	138	166	CWC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
	6SG	175	89	CWC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
	6FP	194	237	CWC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
	8SG	120	221	LGCC	LGCC	0	0	NR	NR	NR	NR	0.00	0.00
	8FP	196	181	LGCC	LGCC	0	0	NR	NR	NR	NR	0.00	0.00
SM-62	ND	144	194	YGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	2SG	194	203	LGCC	LGCC	0	0	NR	NR	NR	NR	0.00	0.00
	2FP	173	112	LGCC	LGCC	0	0	NR	NR	NR	NR	0.00	0.00

Contd..

	4FP	191	201	CWC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
	6SG	109	225	CWC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
	6FP	119	193	CWC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
	8SG	204	139	CWC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
	8FP	197	147	FW	FW	0	0	NR	NR	NR	NR	0.00	0.00
SM-68	ND	165	225	YGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	2SG	97	220	FWB	FW	0	0	NR	NR	NR	NR	0.00	0.00
	2FP	207	183	FW	FW	0	0	NR	NR	NR	NR	0.00	0.00
	4SG	211	183	YGCC	FW	0	0	NR	NR	NR	NR	0.00	0.00
	4FP	193	219	FW	FW	0	0	NR	NR	NR	NR	0.00	0.00
	6SG	126	134	LGCC	FW	0	0	NR	NR	NR	NR	0.00	0.00
	6FP	196	173	CWC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
	8S	189	174	FBW	FBW	0	0	NR	NR	NR	NR	0.00	0.00
	8FP	172	163	CWC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
SM-69	ND	162	224	LGCC	LGCC	0	0	NR	NR	NR	NR	0.00	0.00
	2SG	163	184	LGCC	LGCC	0	0	NR	NR	NR	NR	0.00	0.00
	2FP	155	115	YGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	4SG	163	135	YGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	4FP	182	145	YGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	6SG	180	128	YGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	6FP	178	165	GCC	GCC	0	0	NR	NR	NR	NR	0.00	0.00
	8SG	190	183	LGCC	LGCC	0	0	NR	NR	NR	NR	0.00	0.00
	8FP	195	121	LGCC	LGCC	0	0	NR	NR	NR	NR	0.00	0.00
SM-70	ND	187	97	LGCC	LGCC	0	0	NR	NR	NR	NR	0.00	0.00
	2SG	173	109	LGCC	LGCC	0	0	NR	NR	NR	NR	0.00	0.00
	2FP	177	134	YGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	4SG	166	142	LGCC	LGCC	0	0	NR	NR	NR	NR	0.00	0.00
	4FP	172	128	LGCC	LGCC	0	0	NR	NR	NR	NR	0.00	0.00
	6SG	192	112	LGCC	LGCC	0	0	NR	NR	NR	NR	0.00	0.00
	6FP	205	110	LGCC	LGCC	0	0	NR	NR	NR	NR	0.00	0.00
	8SJ	202	132	YGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	8FP	183	165	CWC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
SM-71	ND	183	164	GCC	GCC	0	0	NR	NR	NR	NR	0.00	0.00
	2SG	146	198	GCC	GCC	0	0	NR	NR	NR	NR	0.00	0.00
	2FP	122	176	GCC	GCC	0	0	NR	NR	NR	NR	0.00	0.00
	4SG	223	134	GCC	GCC	0	0	NR	NR	NR	NR	0.00	0.00
	4FP	193	122	GCC	GCC	0	0	NR	NR	NR	NR	0.00	0.00
	6SG	203	165	GCC	GCC	0	0	NR	NR	NR	NR	0.00	0.00
	6FP	195	143	YGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	8SG	175	105	YGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	8FP	254	162	YGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
SM-72	ND	194	134	YGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	2SG	243	138	GCC	GCC	0	0	NR	NR	NR	NR	0.00	0.00
	2FP	274	124	GCC	GCC	0	0	NR	NR	NR	NR	0.00	0.00
	4SG	185	136	GCC	GCC	10	7	Good	Good	GSEM	GSEM	5.41	5.15
	4FP	199	173	GCC	GCC	11	6	Good	Good	GSEM	GSEM	5.53	3.47

Contd..

	6SG	221	198	GCC	GCC	0	0	NR	NR	NR	NR	0.00	0.00
	6FP	221	186	GCC	GCC	0	0	NR	NR	NR	NR	0.00	0.00
	8SG	264	156	GCC	GCC	0	0	NR	NR	NR	NR	0.00	0.00
	8FP	283	144	GCC	GCC	0	0	NR	NR	Rz	Rz	0.00	0.00
SM-73	ND	193	133	LGCC	LGCC	0	0	NR	NR	NR	NR	0.00	0.00
	2SG	194	134	YGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	2FP	134	164	YGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	4SG	174	185	YGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	4FP	173	209	YGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	6SG	194	126	CWC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
	6FP	148	104	CWC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
	8SG	284	159	CWC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
	8FP	194	98	CWC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
SM-74	ND	183	183	FBW	FBW	0	0	NR	NR	NR	NR	0.00	0.00
	2SG	175	182	LGCC	LGCC	0	0	NR	NR	NR	NR	0.00	0.00
	2FP	174	137	LGCC	LGCC	0	0	NR	NR	NR	NR	0.00	0.00
	4SG	193	133	LGCC	LGCC	3	0	Good	NR	GSEM	NR	1.55	0.00
	4FP	173	148	LGCC	LGCC	0	0	NR	NR	NR	NR	0.00	0.00
	6SG	137	129	FBW	FBW	0	0	NR	NR	NR	NR	0.00	0.00
	6FP	133	192	FBW	FBW	0	0	NR	NR	NR	NR	0.00	0.00
	8SG	123	176	FBW	FBW	0	0	NR	NR	NR	NR	0.00	0.00
	8FP	175	163	FBW	FBW	0	0	NR	NR	NR	NR	0.00	0.00
SM-75	ND	191	138	LGCC	LGCC	4	0	NR	Good	NR	GSEM	2.09	0.00
	2SG	243	137	GCC	YGCC	7	4	Good	Good	GSEM	GSEM	2.88	2.92
	2FP	209	152	YGCC	YGCC	7	5	Good	Good	GSEM	GSEM	3.35	3.29
	4SG	215	141	GCC	YGCC	6	6	Good	Good	GSEM	GSEM	2.79	4.26
	4FP	146	179	YGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	6SG	219	167	YGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	6FP	220	155	YGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	8SG	172	157	LGCC	LGCC	0	0	NR	NR	NR	NR	0.00	0.00
	8FP	141	138	LGCC	LGCC	0	0	NR	NR	NR	NR	0.00	0.00
SM-76	ND	272	173	GCC	GCC	0	0	NR	NR	NR	NR	0.00	0.00
	2SG	178	126	YGCC	FBW	10	0	Good	NR	GSEM	NR	5.62	0.00
	2FP	192	184	FW	FBW	0	0	NR	NR	NR	NR	0.00	0.00
	4SG	183	172	FBW	FBW	0	0	NR	NR	NR	NR	0.00	0.00
	4FP	193	160	FBW	FBW	0	0	NR	NR	NR	NR	0.00	0.00
	6SG	176	168	FBW	FBW	0	0	NR	NR	NR	NR	0.00	0.00
	6FP	158	148	FBW	FBW	0	0	NR	NR	NR	NR	0.00	0.00
	8SG	83	125	FBW	FBW	0	0	NR	NR	NR	NR	0.00	0.00
	8FP	273	153	FBW	FBW	0	0	NR	NR	NR	NR	0.00	0.00
SM-77	ND	187	97	LGCC	LGCC	0	0	NR	NR	NR	NR	0.00	0.00
	2SG	224	172	YGCC	WCC	0	0	NR	NR	NR	NR	0.00	0.00
	2FP	186	185	CWC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
	4SG	188	164	CWC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
	4FP	153	140	CWC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
	6SG	151	131	CWC	CWC	0	0	NR	NR	NR	NR	0.00	0.00

Contd..

	6FP	170	173	CWC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
	8SG	164	168	CWC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
	8FP	179	209	CWC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
SM-78	ND	162	224	LGCC	LGCC	0	0	NR	NR	NR	NR	0.00	0.00
	2SG	184	116	YGCC	YGCC	9	0	Good	NR	GSEM	NR	4.89	0.00
	2FP	201	212	GCC	GCC	0	0	NR	NR	NR	NR	0.00	0.00
	4SG	236	169	LGCC	LGCC	0	0	NR	NR	NR	NR	0.00	0.00
	4FP	223	145	LGCC	LGCC	0	0	NR	NR	NR	NR	0.00	0.00
	6SG	172	103	LGCC	LGCC	0	0	NR	NR	NR	NR	0.00	0.00
	6FP	184	115	YGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	8SG	284	127	LGCC	LGCC	0	0	NR	NR	NR	NR	0.00	0.00
	8FP	179	147	LGCC	LGCC	0	0	NR	NR	NR	NR	0.00	0.00
SM-79	ND	185	123	YGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	2SG	230	183	LGCC	LGCC	4	0	Good	NR	GSEM	NR	1.74	0.00
	2FP	233	190	LGCC	LGCC	0	0	NR	NR	NR	NR	0.00	0.00
	4SG	216	149	YGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	4FP	184	182	CWC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
	6SG	144	164	GCC	GCC	0	0	NR	NR	NR	NR	0.00	0.00
	6FP	162	175	GCC	GCC	0	0	NR	NR	NR	NR	0.00	0.00
	8SG	173	231	GCC	GCC	0	0	NR	NR	NR	NR	0.00	0.00
	8FP	184	273	GCC	GCC	0	0	NR	NR	NR	NR	0.00	0.00
SM-80	ND	194	134	YGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	2SG	195	195	GCC	GCC	0	0	NR	NR	NR	NR	0.00	0.00
	2FP	195	188	YGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	4SG	248	193	YGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	4FP	127	183	YGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	6SG	234	175	GCC	GCC	0	0	NR	NR	NR	NR	0.00	0.00
	6FP	173	237	GCC	GCC	0	0	NR	NR	NR	NR	0.00	0.00
	8SG	172	254	GCC	GCC	10	6	Good	Good	GSEM	GSEM	5.81	2.36
	8FP	234	28	GCC	GCC	0	0	NR	NR	NR	NR	0.00	0.00
SM-81	ND	188	164	CWC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
	2SG	126	191	GCC	GCC	11	0	good	NR	GSEM	NR	8.73	0.00
	2FP	272	173	GCC	GCC	0	0	NR	NR	NR	NR	0.00	0.00
	4SG	284	253	GCC	GCC	0	0	NR	NR	NR	NR	0.00	0.00
	4FP	193	184	GCC	GCC	0	0	NR	NR	Rz	Rz	0.00	0.00
	6SG	204	174	YGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	6FP	193	168	YGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	8SG	185	123	YGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	8FP	149	173	YGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
SM-82	ND	173	183	GCC	GCC	0	0	NR	NR	NR	NR	0.00	0.00
	2SG	137	182	CWC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
	2FP	133	161	CWC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
	4SG	170	107	CWC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
	4FP	172	243	CWC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
	6SG	140	264	LGCC	LGCC	0	0	NR	NR	NR	NR	0.00	0.00
	6FP	138	180	LGCC	LGCC	0	0	NR	NR	NR	NR	0.00	0.00

Contd..

	8SG	131	189	LGCC	LGCC	0	0	NR	NR	NR	NR	0.00	0.00
	8FP	183	172	LGCC	LGCC	0	0	NR	NR	NR	NR	0.00	0.00
SM-83	ND	175	118	CWC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
	2SG	184	166	GCC	FBW	3	0	Good	NR	GSEM	NR	1.63	0.00
	2FP	194	47	FBW	FBW	9	0	NR	NR	NR	NR	4.64	0.00
	4SG	120	143	LGCC	FBW	4	0	Good	NR	GSEM	NR	3.33	0.00
	4FP	112	175	FBW	FBW	0	0	NR	NR	NR	NR	0.00	0.00
	6SG	220	183	FBW	FBW	0	0	NR	NR	NR	NR	0.00	0.00
	6FP	211	108	FBW	FBW	0	0	NR	NR	NR	NR	0.00	0.00
	8SG	212	165	FBW	FBW	0	0	NR	NR	NR	NR	0.00	0.00
	8FP	220	172	FBW	FBW	0	0	NR	NR	NR	NR	0.00	0.00
SM-84	ND	166	173	GCC	GCC	2	1	Good	Good	GSEM	GSEM	1.16	0.52
	2SG	162	184	FBW	FBW	9	6	Good	Good	GSEM	GSEM	4.66	3.11
	2FP	173	197	FW	FW	0	0	NR	NR	NR	NR	0.00	0.00
	4SG	212	195	FBW	FBW	0	0	NR	NR	NR	NR	0.00	0.00
	4FP	78	164	FBW	FBW	0	0	NR	NR	NR	NR	0.00	0.00
	6SG	94	181	YGCC	YGCC	4	2	Good	Good	GSEM	GSEM	4.26	1.10
	6FP	183	178	YGCC	YGCC	7	7	Good	Good	GSEM	GSEM	3.83	3.93
	8SG	193	193	YGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	8FP	243	182	YGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
SM-85	ND	195	195	GCC	GCC	0	0	NR	NR	NR	NR	0.00	0.00
	2SG	274	184	GCC	YGCC	0	0	NR	NR	GSEM	NR	0.00	0.00
	2FP	185	154	YGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	4SG	199	171	LGCC	LGCC	0	0	NR	NR	NR	NR	0.00	0.00
	4FP	221	149	LGCC	LGCC	0	0	NR	NR	NR	NR	0.00	0.00
	6SG	221	236	FBW	FBW	0	0	NR	NR	NR	NR	0.00	0.00
	6FP	264	199	FBW	FBW	0	0	NR	NR	NR	NR	0.00	0.00
	8SG	283	176	FBW	FBW	0	0	NR	NR	NR	NR	0.00	0.00
	8FP	194	196	FBW	FBW	0	0	NR	NR	NR	NR	0.00	0.00
SM-86	ND	194	134	YGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	2SG	134	188	FBW	FBW	4	0	Good	NR	GSEM	NR	2.99	0.00
	2FP	174	192	FBW	FBW	0	0	NR	NR	NR	NR	0.00	0.00
	4SG	173	182	FBW	FBW	0	0	NR	NR	NR	NR	0.00	0.00
	4FP	194	120	FBW	FBW	0	0	NR	NR	NR	NR	0.00	0.00
	6SG	148	111	CWC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
	6FP	284	148	CWC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
	8SG	194	128	CWC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
	8FP	175	172	CWC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
SM-87	ND	173	183	GCC	GCC	0	0	NR	NR	NR	NR	0.00	0.00
	2SG	174	184	YGCC	WCC	4	0	Good	NR	GSEM	NR	2.30	0.00
	2FP	193	198	CWC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
	4SG	173	138	LGCC	CWC	2	0	Good	NR	GSEM	NR	1.16	0.00
	4FP	137	146	CWC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
	6SG	133	133	CWC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
	6FP	123	102	CWC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
	8SG	175	118	CWC	CWC	0	0	NR	NR	NR	NR	0.00	0.00

Contd..

	8FP	193	127	CWC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
SM-88	ND	187	97	LGCC	LGCC	0	0	NR	NR	NR	NR	0.00	0.00
	2SG	239	145	CWC	CWC	2	0	Good	NR	GSEM	NR	0.84	0.00
	2FP	184	132	CWC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
	4SG	128	184	LGCC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
	4FP	172	235	CWC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
	6SG	184	137	CWC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
	6FP	198	121	FW	FW	0	0	NR	NR	NR	NR	0.00	0.00
	8SG	138	172	FW	FW	0	0	NR	NR	NR	NR	0.00	0.00
	8FP	146	204	FW	FW	0	0	NR	NR	NR	NR	0.00	0.00
SM-89	ND	183	102	GCC	GCC	0	0	NR	NR	NR	NR	0.00	0.00
	2SG	133	114	FW	FW	0	0	NR	NR	NR	NR	0.00	0.00
	2FP	102	173	FW	FW	0	0	NR	NR	NR	NR	0.00	0.00
	4SG	118	183	FW	FW	0	0	NR	NR	NR	NR	0.00	0.00
	4FP	127	193	CWC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
	6SG	145	124	GCC	GCC	6	3	Good	Good	GSEM	GSEM	4.14	2.42
	6FP	132	127	GCC	GCC	0	0	NR	NR	NR	NR	0.00	0.00
	8SG	184	113	GCC	GCC	0	0	NR	NR	NR	NR	0.00	0.00
	8FP	235	194	YGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
SM-90	ND	284	253	GCC	GCC	0	0	NR	NR	NR	NR	0.00	0.00
	2SG	137	191	FBW	FBW	0	0	NR	NR	NR	NR	0.00	0.00
	2FP	121	187	YGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	4SG	172	183	FBW	FBW	0	0	NR	NR	NR	NR	0.00	0.00
	4FP	204	165	FBW	FBW	0	0	NR	NR	NR	NR	0.00	0.00
	6SG	114	13	GCC	GCC	0	0	Rz	Rz	Rz	Rz	0.00	0.00
	6FP	173	183	GCC	GCC	0	0	NR	NR	NR	NR	0.00	0.00
	8SG	183	194	FBW	FBW	0	0	NR	NR	NR	NR	0.00	0.00
	8FP	193	137	LGCC	LGCC	0	0	NR	NR	NR	NR	0.00	0.00
SM-91	ND	162	224	LGCC	LGCC	0	0	NR	NR	NR	NR	0.00	0.00
	2SG	124	213	FBW	FBW	0	0	NR	NR	NR	NR	0.00	0.00
	2FP	127	193	FBW	FBW	0	0	NR	NR	NR	NR	0.00	0.00
	4SG	113	93	FW	FW	0	0	NR	NR	NR	NR	0.00	0.00
	4FP	194	192	FW	FW	0	0	NR	NR	NR	NR	0.00	0.00
	6SG	191	173	FBW	FBW	0	0	NR	NR	NR	NR	0.00	0.00
	6FP	102	182	FBW	FBW	0	0	NR	NR	NR	NR	0.00	0.00
	8SG	118	163	YGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	8FP	127	166	LGCC	LGCC	0	0	NR	NR	NR	NR	0.00	0.00
SM-92	ND	195	195	GCC	GCC	0	0	NR	NR	NR	NR	0.00	0.00
	2SG	145	154	LGCC	LGCC	8	0	Good	NR	GSEM	NR	5.52	0.00
	2FP	132	168	LGCC	LGCC	0	0	NR	NR	NR	NR	0.00	0.00
	4SG	184	187	FBW	FBW	0	0	NR	NR	NR	NR	0.00	0.00
	4FP	235	121	FBW	FBW	0	0	NR	NR	NR	NR	0.00	0.00
	6SG	137	209	LGCC	LGCC	0	0	NR	NR	NR	NR	0.00	0.00
	6FP	121	211	LGCC	LGCC	0	0	NR	NR	NR	NR	0.00	0.00
	8SG	172	217	LGCC	LGCC	0	0	NR	NR	NR	NR	0.00	0.00
	8FP	204	183	LGCC	LGCC	0	0	NR	NR	NR	NR	0.00	0.00

Contd..

SM-93	ND	194	134	YGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	2SG	114	224	GCC	FBW	0	0	Good	NR	GSEM	NR	0.00	0.00
	2FP	173	93	FBW	FBW	0	0	NR	NR	NR	NR	0.00	0.00
	4SG	183	190	LFW	LFW	0	0	NR	NR	NR	NR	0.00	0.00
	4FP	193	92	FBW	FBW	10	6	Good	Good	GSEM	GSEM	5.18	6.52
	6SG	124	124	FBW	FBW	0	0	NR	NR	NR	NR	0.00	0.00
	6FP	127	225	LGCC	LGCC	0	0	NR	NR	NR	NR	0.00	0.00
	8SG	113	184	GCC	GCC	0	0	NR	NR	NR	NR	0.00	0.00
	8FP	194	190	YGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
SM-94	ND	185	123	YGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	2SG	191	218	YGCC	YGCC	4	0	Good	NR	GSEM	NR	2.09	0.00
	2FP	187	97	LGCC	LGCC	0	0	NR	NR	NR	NR	0.00	0.00
	4SG	183	165	FBW	FBW	0	0	NR	NR	NR	NR	0.00	0.00
	4FP	165	183	FBW	FBW	0	0	NR	NR	NR	NR	0.00	0.00
	6SG	13	194	YGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	6FP	183	199	YGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	8SG	194	219	LGCC	LGCC	0	0	NR	NR	NR	NR	0.00	0.00
	8FP	137	172	YGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
SM-95	ND	183	102	GCC	GCC	0	0	NR	NR	NR	NR	0.00	0.00
	2SG	213	211	GCC	GCC	0	0	NR	NR	NR	NR	0.00	0.00
	2FP	193	234	YGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	4SG	93	201	YGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	4FP	192	148	YGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	6SG	173	183	LGCC	LGCC	0	0	NR	NR	NR	NR	0.00	0.00
	6FP	182	191	LGCC	LGCC	0	0	NR	NR	NR	NR	0.00	0.00
	8SG	163	193	YGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	8FP	166	142	GCC	GCC	0	0	NR	NR	NR	NR	0.00	0.00
SM-96	ND	194	134	YGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	2SG	154	183	YGCC	YGCC	3	0	Good	NR	GSEM	NR	1.95	0.00
	2FP	168	192	LGCC	LGCC	8	0	NR	NR	NR	NR	4.76	0.00
	4SG	187	184	GCC	FBW	9	0	Good	NR	GSEM	NR	4.81	0.00
	4FP	121	194	FBW	FBW	0	0	NR	NR	NR	NR	0.00	0.00
	6SG	209	175	LFW	LFW	0	0	NR	NR	NR	NR	0.00	0.00
	6FP	211	173	FBW	FBW	0	0	NR	NR	NR	NR	0.00	0.00
	8SG	217	154	FBW	FBW	0	0	NR	NR	NR	NR	0.00	0.00
	8FP	183	166	GCC	GCC	0	0	NR	NR	NR	NR	0.00	0.00
SM-97	ND	165	225	YGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	2SG	224	89	FBW	FBW	0	0	NR	NR	NR	NR	0.00	0.00
	2FP	93	237	LFW	LFW	0	0	NR	NR	NR	NR	0.00	0.00
	4SG	190	221	FBW	FBW	0	0	NR	NR	NR	NR	0.00	0.00
	4FP	92	181	FBW	FBW	0	0	NR	NR	NR	NR	0.00	0.00
	6SG	124	203	FBW	FBW	0	0	NR	NR	NR	NR	0.00	0.00
	6FP	225	112	FBW	FBW	0	0	NR	NR	NR	NR	0.00	0.00
	8SG	184	125	LGCC	LGCC	0	0	NR	NR	NR	NR	0.00	0.00
	8FP	190	201	LGCC	LGCC	0	0	NR	NR	NR	NR	0.00	0.00
SM-98	ND	272	173	GCC	GCC	0	0	NR	NR	NR	NR	0.00	0.00

Contd..

	2SG	218	225	LGCC	LGCC	0	0	Rz	Rz	Rz	Rz	0.00	0.00
	2FP	97	193	YGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	4SG	165	139	FBW	FBW	0	0	NR	NR	NR	NR	0.00	0.00
	4FP	183	147	FBW	FBW	0	0	NR	NR	NR	NR	0.00	0.00
	6SG	194	220	YGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	6FP	199	183	YGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	8SG	219	183	YGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	8FP	172	219	YGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
SM-99	ND	185	123	YGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	2SG	211	134	LGCC	LGCC	0	0	NR	NR	NR	NR	0.00	0.00
	2FP	234	173	LGCC	LGCC	0	0	NR	NR	NR	NR	0.00	0.00
	4SG	201	174	FBW	FBW	0	0	NR	NR	NR	NR	0.00	0.00
	4FP	148	163	FBW	FBW	0	0	NR	NR	NR	NR	0.00	0.00
	6SG	183	184	YGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	6FP	191	138	LGCC	LGCC	4	0	NR	Good	NR	GSEM	2.09	0.00
	8SG	193	146	GCC	GCC	0	0	NR	NR	NR	NR	0.00	0.00
	8FP	142	133	GCC	GCC	0	0	NR	NR	NR	NR	0.00	0.00
SM-100	ND	284	253	GCC	GCC	0	0	NR	NR	NR	NR	0.00	0.00
	2SG	183	102	GCC	GCC	0	0	NR	NR	NR	NR	0.00	0.00
	2FP	192	118	YGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	4SG	184	127	FBW	FBW	0	0	NR	NR	NR	NR	0.00	0.00
	4FP	194	145	LFW	LFW	0	0	NR	NR	NR	NR	0.00	0.00
	6SG	175	132	FBW	FBW	0	0	NR	NR	NR	NR	0.00	0.00
	6FP	173	184	FBW	FBW	0	0	NR	NR	NR	NR	0.00	0.00
	8S	154	235	YGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	8FP	166	137	GCC	GCC	0	0	NR	NR	NR	NR	0.00	0.00
SM-101	ND	137	209	LGCC	LGCC	0	0	NR	NR	NR	NR	0.00	0.00
	2SG	89	121	LFW	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	2FP	237	172	CWC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
	4SG	221	204	CWC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
	4FP	181	114	FBW	FBW	0	0	NR	NR	NR	NR	0.00	0.00
	6SG	203	173	LFW	LFW	0	0	NR	NR	NR	NR	0.00	0.00
	6FP	112	183	YGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	8SG	125	193	GCC	GCC	0	0	NR	NR	NR	NR	0.00	0.00
	8FP	201	124	GCC	GCC	0	0	NR	NR	NR	NR	0.00	0.00
SM-102	ND	162	224	LGCC	LGCC	0	0	NR	NR	NR	NR	0.00	0.00
	2SG	225	127	GCC	GCC	0	0	NR	NR	NR	NR	0.00	0.00
	2FP	193	113	GCC	GCC	0	0	NR	NR	NR	NR	0.00	0.00
	4SG	139	194	YGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	4FP	147	191	LGCC	LGCC	0	0	NR	NR	NR	NR	0.00	0.00
	6SG	220	187	FBW	FBW	0	0	NR	NR	NR	NR	0.00	0.00
	6FP	183	183	FW	FW	0	0	NR	NR	NR	NR	0.00	0.00
	8SJ	183	165	FBW	FBW	0	0	NR	NR	NR	NR	0.00	0.00
	8FP	219	13	FBW	FBW	0	0	NR	NR	NR	NR	0.00	0.00
SM-103	ND	194	134	YGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	2SG	134	183	FBW	FBW	0	0	NR	NR	NR	NR	0.00	0.00

Contd..

	2FP	173	194	YGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	4SG	174	137	YGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	4FP	163	213	LGCC	LGCC	0	0	NR	NR	NR	NR	0.00	0.00
	6SG	184	193	GCC	GCC	0	0	NR	NR	NR	NR	0.00	0.00
	6FP	115	93	GCC	GCC	0	0	NR	NR	NR	NR	0.00	0.00
	8SG	135	192	YGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	8FP	145	173	YGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
SM-104	ND	183	183	FBW	FBW	0	0	NR	NR	NR	NR	0.00	0.00
	2SG	128	182	YGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	2FP	165	163	GCC	GCC	0	0	NR	NR	NR	NR	0.00	0.00
	4SG	183	166	FBW	FBW	0	0	NR	NR	NR	NR	0.00	0.00
	4FP	148	154	GCC	GCC	0	0	NR	NR	NR	NR	0.00	0.00
	6SG	183	168	LGCC	LGCC	0	0	NR	NR	NR	NR	0.00	0.00
	6FP	191	187	LFW	LFW	0	0	NR	NR	NR	NR	0.00	0.00
	8SG	193	121	LFW	LFW	0	0	NR	NR	NR	NR	0.00	0.00
	8FP	142	209	YGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
SM-105	ND	142	181	GCC	GCC	0	0	NR	NR	NR	NR	0.00	0.00
	2SG	183	211	YGCC	YGCC	5	0	Good	NR	GSEM	NR	2.73	0.00
	2FP	192	217	LGCC	LGCC	0	0	NR	NR	NR	NR	0.00	0.00
	4SG	184	183	FBW	FBW	0	0	NR	NR	NR	NR	0.00	0.00
	4FP	194	224	FBW	FBW	0	0	NR	NR	NR	NR	0.00	0.00
	6SG	175	93	FBW	FBW	0	0	NR	NR	NR	NR	0.00	0.00
	6FP	173	190	LFW	LFW	0	0	NR	NR	NR	NR	0.00	0.00
	8SG	154	92	GCC	GCC	0	0	NR	NR	NR	NR	0.00	0.00
	8FP	166	124	YGCC	YGCC	0	0	NR	NR	Rz	Rz	0.00	0.00
SM-106	ND	139	194	YGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	2SG	89	225	YGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	2FP	237	184	LGCC	LGCC	0	0	NR	NR	NR	NR	0.00	0.00
	4SG	221	190	LGCC	LGCC	0	0	NR	NR	NR	NR	0.00	0.00
	4FP	181	218	FBW	FBW	0	0	NR	NR	NR	NR	0.00	0.00
	6SG	203	97	FW	FW	0	0	NR	NR	NR	NR	0.00	0.00
	6FP	112	165	CWC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
	8SG	125	183	CWC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
	8FP	201	194	LGCC	LGCC	0	0	NR	NR	NR	NR	0.00	0.00
SM-107	ND	183	183	FBW	FBW	0	0	NR	NR	NR	NR	0.00	0.00
	2SG	225	199	GCC	FBW	0	0	NR	NR	NR	NR	0.00	0.00
	2FP	193	219	FW	FW	0	0	NR	NR	NR	NR	0.00	0.00
	4SG	139	172	FBW	FBW	0	0	NR	NR	NR	NR	0.00	0.00
	4FP	147	211	CWC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
	6SG	220	234	LGCC	LGCC	0	0	NR	NR	NR	NR	0.00	0.00
	6FP	183	201	YGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	8SG	183	148	YGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	8FP	219	183	YGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
SM-108	ND	183	102	GCC	GCC	0	0	NR	NR	NR	NR	0.00	0.00
	2SG	134	191	YGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	2FP	173	193	GCC	GCC	0	0	NR	NR	NR	NR	0.00	0.00

Contd..

	4SG	174	142	LGCC	LGCC	0	0	NR	NR	NR	NR	0.00	0.00
	4FP	163	183	LGCC	LGCC	0	0	NR	NR	NR	NR	0.00	0.00
	6SG	184	192	LGCC	LGCC	0	0	NR	NR	NR	NR	0.00	0.00
	6FP	115	184	YGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	8SG	135	194	LGCC	LGCC	0	0	NR	NR	NR	NR	0.00	0.00
	8FP	145	175	LGCC	LGCC	0	0	NR	NR	NR	NR	0.00	0.00
SM-109	ND	194	134	YGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	2SG	128	173	LGCC	LGCC	0	0	NR	NR	NR	NR	0.00	0.00
	2FP	165	154	LGCC	LGCC	0	0	NR	NR	NR	NR	0.00	0.00
	4SG	183	166	YGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	4FP	121	89	CWC	CWC	0	0	NR	NR	NR	NR	0.00	0.00
	6SG	109	237	GCC	GCC	0	0	NR	NR	NR	NR	0.00	0.00
	6FP	134	221	GCC	GCC	0	0	NR	NR	NR	NR	0.00	0.00
	8SG	142	181	GCC	GCC	0	0	NR	NR	NR	NR	0.00	0.00
	8FP	128	203	GCC	GCC	0	0	NR	NR	NR	NR	0.00	0.00
SM110	ND	187	182	FBW	FBW	3	0	Good	NR	GSEM	NR	1.60	0.00
	2SG	112	112	GCC	GCC	0	0	Good	Good	GSEM	GSEM	8.96	2.04
	2FP	110	125	YGCC	YGCC	0	0	Good	Good	GSEM	GSEM	0.00	0.00
	4SG	132	201	YGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	4FP	165	225	YGCC	YGCC	0	0	NR	NR	NR	NR	0.00	0.00
	6SG	198	193	GCC	GCC	0	0	NR	NR	NR	NR	0.00	0.00
	6FP	176	139	GCC	GCC	0	0	NR	NR	NR	NR	0.00	0.00
	8SG	134	147	GCC	GCC	12	3	NR	NR	NR	NR	0.00	0.00
	8FP	122	220	GCC	GCC	11	7	NR	NR	NR	NR	0.00	0.00

Where,

SM- Somatic embryogenesis medium

SG- Silica gel (Self indicating blue)

GCC- Green colour callus

YGCC- Yellowish Green colour callus

FWB- Friable whitish brown colour callus

LFW- Light friable watery callus

GSEM- Globular Stage somatic embryo

ND-Non desiccated

FP- Filter Paper

CWC- Compact White callus

LGCC- Light green colour callus

FW- Friable watery colour callus

NR- No response

HSEM- Heart stage somatic embryo

CHAPTER V
SUMMARY AND CONCLUSION

In the present investigation entitled "Effect of Desiccation on Callus Cultures in Cotton". Two explants viz. hypocotyls and leaf bits of *G .hirsutum* cotton variety PKV-Rajat were used for callus induction, desiccation and somatic embryogenesis responses in cotton.

i) Minimum numbers of days (11.94 + 0.10) were recorded for callus initiation in hypocotyls and (24.74 ± 0.22) for leaf bits on medium CM10 (5 mg/l NAA + 0.5 mg/L BAP).

ii) The highest percentage of callusing (86.40%) was produced by hypocotyls and 79% for leaf bits in medium CM-11 (10 mg/l NAA + 1 mg/l BAP)

iii) Rhizogenesis was observed in both of the explants with leaf bits and hypocotyls after 15 days of inoculation on CM-2 medium (MS + 3 mg/l NAA + 0.1 mg/l IAA).

iv) In case of hypocotyls callus was multiplied maximum (243.83 ± 0.50 %), where as in case of leaf bits (230.22 ± 0.11%) on M3 (5 mg/l NAA + 0.5 mg/l BAP).

v) Desiccation percentage was recorded after two, four, six and eight hr and they were 11.78, 18, 22.85 and 28.26% respectively in silica gel. However, it was observed higher 44.53, 49.82, 52.92, and 59% in filter paper desiccation.

Higher percentage of moisture loss was also observed in the new callus as compared to old callus during desiccation treatment.

vi) The relative growth rate of callus was highest in hypocotyls (440.00 ± 0.54%) after 8 hrs desiccation on filter paper and leaf bits it was 425.67 ± 0.31 per cent. The rate of desiccation is faster in filter paper than silica gel desiccation.

vii) The maximum percentage of globular somatic embryos (13.39%) was observed in hypocotyls than leaf bits (11.34%).Heart shape

somatic embryos were also formed in hypocotyls and leaf bits explants having frequency 2.99 and 2.92% on SM-53 (MS + 0.2 mg/l kin) using silica gel desiccation.

viii) Filter paper desiccated calli beared globular stage somatic embryos up to (9.87%) in hypocotyls on medium SM-47 (2mg/l NAA+ 2mg/l BAP) and leaf bits (6.67%) on media SM-23 (0.5mg/l NAA 0.5mg/l BAP).

ix) Non desiccated calli beared globular somatic embryos up to 2.41% in hypocotyls and leaf bits 1.73% on medium SM-59(MS+1mg/l BAP).

Conclusions

i. The rate of desiccation was observed highest in filter paper than silica gel desiccation and similar trend was also observed for callus multiplication after desiccation

ii. Silica gel desiccation treatment produced more globular somatic embryos than filter paper desiccation treatment. It was also observed heart shaped somatic embryos were produced from 2h silica gel desiccation however no response in filter paper.

iii. Frequency of globular somatic embryos was more in two and four hour Silica gel desiccators than filter paper and non desiccated callus.

iv. Hypocotyl explants and silica gel desiccation was for 2 and 4 h found effective for induction of somatic embryogenesis than leaf bit explants and filter paper desiccation.

CHAPTER – VI

IMPLICATIONS

In the present investigation entitled "Effect of desiccation on callus cultures in cotton." concluded that the hypocotyls explants found suitable for callus induction. Desiccation treatment using silica gel will help to improve conversion of globular somatic embryos in to heart shaped somatic embryos. It helps to standardize protocol of somatic embryogenesis in cotton variety PKV- Rajat.

CHAPTER VI

LITERATURE CITED

Anonymous. 2006. www.iimahd.ernet.in/ publication data.vgandhi.pdf

Anonymous. 2009: Advisory Board -2009, Area, production and productivity of cotton. WWW. cotcrop.gov.in/statistics.asp. Cotton

*Aydn, Y., Z. Ipekc, T. Talas-orgas, A.Zehr, K. Bajrovc and N. Gozukrmz, 2004: High frequency somatic embryogenesis in cotton, Biologia-Plantarum, 48 (4): 491-495.

*Bajaj, Y.P.S., 1990b. Somaclonal variation in crop improvement (ed). Biotechnology in Agriculture and forestry, 1 Springer – verlag Berlin 11: 1-685

*Bajaj, Y.P.S., 1995: Somatic embryogenesis and its application for crop improvement In Bajaj, Y.P.S. (ed) Somatic embryogenesis and synthetic seed I, Biotechnology in Agriculture and Forestry 30. Springer, Berlin Heiden, New York: 105-125.

Benedict J.H. and Altman D W 2001.Commercialization of trasgenicc cotton expressing insectisidal crystal protein. In: Jenkins J N, Saha (eds) Genetic improvement of cotton. USDA-ARS. Oxford & IBH,New Delhi, pp.136-201.

*Brar, K.S., B.S.Sandhu and S.S. Gosal, 1998: Tissue culture response of cultivated and wild cotton species. Crop Improvement 25 (1): 59-65.

*Cai-Xiao Ning, Wu Jing Yin and She Jian Ming, 1997: Embryogenic callus induction and plant regeneration in upland cotton. Jiagsu J. Agricultural sciences, 13(4): 225-230.

Carlson, P.S., H.H. Smith and R.D. Dearing, 1972: Parasexual interspecific plant hybridization. Pros. Natl. Acad. Sci. USA, 69: 2292-2294.

Carman J.G. Improved somatic embryogenesis in wheat by partial simulation of the in-ovulo oxygen, growth-regulator and desiccation environments.Planta 175: 417-428.

*Carvalho, J. M., F.C., E. Gonzalez Benito, C. Perez and J.W. dosSantos, 1998: Revista de Oleaginosase Fibrosas 2(1): 13-19.

Chand,S. and A.K. Sahrawat, 2001.Stimulatory effect of partial desiccation on plant regeneration in Indica, rice (*orya Sativa L.*).Plant Biochem. Biotechnology. 10 (1): 43 – 47

*Davidonis, G.H. and R.H. Hamilton, 1983: Plant regeneration from callus cultures of *Gossypium hirsutum L.* Cotton Plant Science Letters, 32: 89-93.

Deng S., Z. Dong, k Zhan, Y. Hu, D Yin and D. Cui 2009: Moderate desiccation dramatically improves shoot regeneration from maize (*Zea mays* L.) callus. *In vitro* Cell. Dev. Biol. Plant 45: 99-103

Desai N.S., P. Suprasanna and V.A. Bapat, 2004: Partial desiccation of embryogenic callus improve plant regeneration frequency in sugarcane (*Saccharum spp.*), J. Plant Biotechnol 6(4): 229-233.

Diah Azria and P.L. Bhalla, 2000.Plant regeneration from mature embryo-derived callus of Australian rice (*Oryza sativa L.*) varieties Aust. J. Agric. Res., 51:305-312

Dunwell,J. M.1981: Influence of genotype and environment on growth of barley embryos *in vitro* .Ann. Bot. 48: 532-542.

*Efe, L., 2005: Callus formation and plant regeneration from two wild cotton species (*Gossypium hirsutum* L and G. *barbadense* L.). Pakistan J. Botany, 37(2): 227-236.

Etienne H, Montoro P, Ferriere M, Carron M.P. 1993: Effect of desiccation, medium osmolarity and Abscisic acid on the maturation of *Hevea brasiliensis* somatic embryos. J. Exp. Bot. 267: 1613-1619.

Evans, D.A. 1989: Samaclonal variation – Genetic basis and breeding application. Trends in Genetics 5: 46-50.

Evans, D.A. and Sharp, W.R. 1986: Somaclonal and gametoclonal, variation D.A. Evans *et al.* (eds), Handbook of plant cell culture, Vol. 4: Techniques and Application Macmillan Publishing company, N.Y., pp. 97-132.

Finer j.j. 1988: Plant regeneration from somatic embryogenesis in many cultivars of cotton (*Gossypium hirsutum* L.), Plant Cell Report 7:481-494.

*Firozabady, E. and D.L. DeBoer, 1993: Plant regeneration via somatic embryogenesis in many cultivars of cotton (*Gossypium hirsutum* L.). *In vitro* Cell Dev. Biol. 29 P: 166-173.

Firozabady, E., D.L. DeBoer, D.J. Merlo, E. L. Halk, L. N. Amerson, K.F. Rashka and E.E. Murray 1987: Transformation of cotton (*Gossypium hirsutum* L.) by *Agrobacterium tumefaciense* and regeneration of transgenic plants. Plant Molecular Biology. 10 P: 105-116.

Fryxell, P.A. (1992): A revised toxonomic interpretation of *Gossypium L.* (Malvaceae) Rheedea 2: 108-165.

*Gautheret, K.J., 1939: Surl possiblilities derealiser a culture indefine des tissues de tuberculus de carotte C.R. Hebd. Sances Acad Sciences 8: 118-120.

Gawel, N. J. and C. D. Robacker 1990: Genetic Control of Somatic embryogenesis in cotton petiole callus culture. Euphytica 49: 249-253

*Gonzalez-Benito, M.E., J.M.F.C., Carvalho and C. Perez, 1997: Somatic embryogenesis of an early cotton cultivar. Pesquisa-Agropecuaria Brasileria 32(5): 485-488.

Gray D.J.1987: Hortical Science 22:810-814.

Guha, S. and S.C. Maheshwari, 1966: Cell division and differentiation of embryos in the pollen grains of Dhatura *in vitro*. Nature 212: 97-98.

Guinn G. 1976: Water deficit and ethylene evoluation by young cotton bolls. Plant physiol. 57: 403-405.

*Guo-YuLong, Li-Ming Yong, Pie-Yan and Cai-yingFan, 1999: Somatic embryogenesis and regenerated plant transplantation in upland cotton (*Gossypium hirsutum* L.). J. Southwest Agricultural University 21(6): 514-517.

*Haberdlandt, G. 1902 : Culture versuche mit isolierten planzenzellen. Sber. Akd. Wiss. Wein III: 69-92.

Hammatt N. Davaeey M.R. 1987: Somatic embryogenesis and plant regeration from cultured zygotic embryos of soybean. Plant Physiol 128: 2191-226.

*Hazra, S., A.V. Kulkarni, A.K. Banerjee, A.B. Dhage, D.C. Agrawal, K.V. Krishnamurthy and S.M. Nalawade, 2002: A rapid and simple method for *In vitro* plant regeneration from split embryo axes of six cultivars of cotton. Biologia – Plantarum, 45 (2): 317-319.

Haslekas C. Starcy R., Nygaard V., Culianz Macia, F. Aalen R. 1998: The expression of a periredoxin antioxidant gene, At perl, in *Arabidopsis thaliana* is seed specific and related to dormancy. Plant Mol. Biol. 36: 833-845.

*Heinz, D.J., H. Krishnamurthy, L.C. Nickell and A. Maretzki, 1979: Cell, tissue and organ culture in Sugarcane Improvement. In Reinert, J. and Y.P.S. Bajaj (eds) Plant Cell, Tissue and Organ Culture Springer verlag, New York: 3-17.

*Higuchi, N. and E. Maeda, 1990: Enhanced plant regeneration in rice callus culture following absisic acids treatment. Jap. J. Crop Sci., 59 (2): 359 – 398.

*Hirimburegama, K., and N. Gamage, 1994.*In vitro* callus and cell cultures of *Gossypium hirsutum* L. (Cotton). The National Science Council of Sri-Lanka 22 (4): 305-312.

Hoemaker,R. C., L. J. Couche and D. W. Galhraith 1986.Characterization of somatic embryogenesis and plant regeneration in cotton. Plant Cell Report.5: 178-181.

*Hussain, S.S., Tayyab Husnain and S. Riazuddin, 2004: Somatic embryo germination and plant development from immature zygotic embryos in cotton.Pakistan J. Biological Sciences, 7 (11) 1946-1949.

Hutchinson,J.B. Silow, R.A. and S.G. Stephens, 1947.The evolution of *Gossypium*.Oxford Univ. Press.

Ikram-Ul-Haq, 2005: Callus proliferation and somatic embryogenesis in cotton (*Gossypium hirsutum* L.), African J. Biotechnology 4(2): 208-209.

Ikram-Ul-Haq and Yusuf Zafar, 2004: High frequency of callus induction its proliferation and somatic embryogenesis in cotton (*Gossypium hirsutum* L.). Plant Biotechnology, 6(1): 55-61.

Jain R.K., S. Jain and R. Wu, 1996.Stimulatory effect of water stress on plant regeration in aromatic Indica rice varieties. Plant cell Report, 15 (6): 449-454.

Kalamani, A., 1994: Initiation of callus and plant regeneration in cotton (*G. hirsutum* L.), Madras Agricultural J. 81(11): 579-580.

*Kohlenbach, N.W., 1966: Die entwick lungs potenzne expantierter and isolierter dauerzeuen. I Das strechanges and tei lungs wanchrstan isolierter Mesophyllzellod Von *macieaya Cordata*. Z. pflanzenphysiol 55 : 142-157.

*Kotte, W. 1922: Kultur versuche mit isolierten warzelspitzen Beitr. Allg Bot 2: 413 –434.

Kumar Shashi and Deepak Pental, 1998: Regeneration of Indian Cotton variety MCV-5 through somatic embryogenesis. Current Sci. 74: 538-540.

*Kouadio, J.Y., M. Kone, Y. Dje, M.A. D. Almeida and M. Zouzou, 2004: Darkness etiolement is a favorable pretreatment to induce somatic embryogenesis from hypocotyls of cotton (*Gossypium hirsutum* L.) Biotechnogie Agronomic, Society et Environment, 8(3): 155-162.

*Kumria, R., V.G. Sunnichan, D.K. Das, S.K. Gupta, V.S. Reddy, R.K. Bhatnagar, and S. Leelavathi, 2003: High frequency somatic embryo production and maturation into normal plants in cotton (*Gossypium hirsutum*) through metabolic stress. Plant Cell Reports 21(7): 635-639.

*Laibach, F., 1925:Das Taubwerden Von Bastardsamen Vnd die Kunstiliche aufzucnt Fruh absterbender Bastarembryonen Z. Bot. 17: 417-459.

Larkin,P.J. and Scowcroft, W.R. 1981: Somaclonal variation - a novel source of variability from cell cultures for plant improvement. Theor. Appl. Genet. 60: 197-214.

Lyon, B. R, Y.L. Cousins, D.J. Liewellyn and E.S. Demmis, 1993: Cotton plant transformed with a bacterial degradation gene are protected from accidental spray drift damaged by the herbicides 2, 4-D, Transgenic Res. 2: 162-169.

Mathews H. Schopke C, Carcamo R, Chanvarriaga P, Fauguet C, Beachy R.N. 1993: Plant Cell Report 12: 328 – 333.

Meichers,G. and Labib, G. (1974).Somatic hybridization of plant by fusion of Protoplasts. 1: Selection of light resistant hybrids of haploid light sensitive varieties of tobacco. Mol. Gen. Genet. 135:277–294.

Merkle S.A., W.A. Parrot and B.S. Flinn (1995). Morphogenesis aspects of somatic embryogenesis. In Thorpe T.A. (ed) *In vitro* embryogenesis in plants. Kluwer, Dordrecht, pp 155 – 203.

Mishra, R., Wang Huai Yu, N.R. Yadav and T.A. Wikins, 2003: Development of a highly regenerable elite Acala cotton (*Gossypium hirsutum*) Cv. axxa – a step towards genotype independent regeneration. Plant Cell, Tissue and Organ Culture, 73 (1): 21-25.

*Mithilesh Kumar and Rakesh Tuli, 2004: Plant regeneration in cotton: a short-term inositol starvation promotes developmental synchrony in somatic embryogenesis. *In vitro* Cellular and Developmental Biology Plant. 40 (3): 294-298.

*Morel, G., 1948: Rechercehs sur la culture associee de parasites obligetories et de tissues vegetaux. Ann.epiphyt. 14:123-234.

Morel, G., 1960: Producing virus free cymbidium. Am. Orchid Soc. Bull. 29: 495-495.

*Morel, G. and C. Martin, 1952: Guerison de dahlias atteints d une maladie a virus C.R.A. Cad. Sci. Paris, 235: 1324-1325.

Murashige, T. and F. Skoog, 1962: A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15: 473-497.

Muthusamy, A. and N. Jayabalan, 2000: *In vitro* regeneration of plantlets from leaf explants of *G. hirsuum* L. J. Indian Soc. Cotton Improv.86-91.

Muthusamy, A., K. Vasanth and N. Jayabalan, 2004: *In vitro* regeneration of cotton plants from shoot tip explants: A simple and alternative method. Pl. Cell Biotechnology and Mol. Biology, 5 (1/2): 15-20.

*Nobecourt, P. 1939: Surlaiperennite et I augmentation de volume des cultures de tissue vegetaus. C. R. Seances Soc. Biol. Ses. Fil. 130:1270-1271.

Nobre, J., D.J. Keith and J.M. Dunwell, 2001: Morphogenesis and regeneration from stomatal guard cell complexes of cotton (*Gossypium hirsutum* L.), Plant Cell Rep. 20(1): 8-15.

Panse, V.G. and P.V. Sukhatme, 1958: Statistical methods for Agricultural workers, ICAR, New Delhi: 48-51.

*Price, H.J. and R.H.Smith, 1979: Somatic embryogenesis in suspension culture of *Gossypium Klostzchianum.* Planta, 145: 305-307.

Parrott, W.A., Dryden. G.,vogt. S., Hilderbanol. D.F., Collins, G.B.,and E.G. Williams, 1988: Optimization of somatic embryogenesis and embryo germination in Soybean. *In vitro* Cell. Dev. Biol. Plant 24: 817 – 821.

*Rajasegar, G., S.R. Rangasamy, P. Venkatachalam, and G. R. Rao, 1996: Callus induction, somatic embryoid formation and plant regeneration in cotton (*Gossypium hirsutum* L.) J. Physiological Research 9 (2): 145-147.

Rajasekaran, K., 1996: Regeneration of plants from cryopreserved embryogenic cell suspension and callus cultures of cotton (*Gossypium hirsutum* L.) Plant Cell Report 15 (11): 859-864.

Rance I.M., W. Tian, Mathews H, de Kochko. A, Beachy R.N., and C. Fauquet (1994).Partial desiccation of mature embryo derived calli, a simple treatment that dramataically enhances the regeneration ability of india rice. Plant cells Rep. 12: 647-651.

Rao Abdul Quygam, S.S. Hussain, N.S. Shahzad, S. Bokhari, A. Yassir, M.S. Raza, A. Rakha, A. Majeed, A.A. Shahid 2005 : Somatic embryogenesis and wild relatives of cotton (*Gossypium* spp.) Journal of Zhejjang University Science B. 7 : 291-298.

*Reinert, J. 1959: Morphogenese und ihre kontrolle on Gewebekuluren aux. Carroten. Naturwissen Schaften. 45: 344-345.

Robbins, W.J. 1922.Effect of autolysed yeast and peptone on growth of excise corn root tips in the dark. Bot. Gaz. (Chicago) 74: 59 – 79.

Saito, T. Nishizawa S. Nishimuras 1991: Improved culture conditions for somatic embryogenesis from *Asparagus officinalis* L. using as aseptic ventilative filter. Plant Cell Report 10: 230 – 234.

Sakhanokho, H.F., A. Zipf, K. Rajasekaran, S.S. Sharma, 2000: Induction of highly embryogenic calli and plant regeneration in upland (*Gossypium hirsutum* L.) and Pima (G. *barbadense* L.) cotton, Crop Science, 41: 1235-1240.

Sakhanokho, H.F., P. Ozias Akins, O.L., May and P.W. Chee, 2004a: Induction of somatic embryogenesis and plant regeneration in select Georgia and Pee Dee Cotton lines. Crop Science 44 (6): 2199-2205.

Sakhanokho, H.F., A. Zipf, K. Rajasekaran, and S.S. Sharma, 2004b: Somatic embryo inititation and germination in diplioid cotton (*Gassypium arboreum* L.) *In vitro* cell.Dev. Biol –plant 40: 177 –1181.

Sakhanokho, H.F., P. Ozias Akins, O.L. May and P.W. Chee, 2005: Putrescine enhances somatic embryogenesis and plant regeneration in upland cotton, Plant Cell, Tissue and Organ Culture, 81 (1): 91-95.

San Noeum, L.H. 1976: Haploids dd. *Hordeum Vulgare* L. parculture *in vitro* non-fecondes. Ann. Amelior. Plantes 26: 751 -754

Scowcroft,W.R. 1977:Somatic cell genetics and plant improvement. Ann. Rev. Agron.29: 59-81.

Scowcroft, W.R., 1984: Genetic variability in tissue culture. Impact on germplasm conservation and utilization. IBPGR, Rome, Italy.

Saharan V; R.C. Yadav, N. Blam, R. Yadav and B.P. Chapagain 2004: High frequency plant regeneration from desiccated calli of indica rice (*Oryza sativa* L.) African Journal of Biotechnology Vol. 3 (5). pp 256-259.

Shepard, J.F., Bidney, D. and Stabin, E, 1980: Potato protoplast in Crop Improvement Science (N.Y.). 208:17-24.

Simpson, D.M. 1954: Department of Agriculture Tech. Bull.Pp.1094.

Skoog, F. and C.O. Miller, 1957: Chemical regulation of growth and organ formation in plant tissue cultured *in vitro*. Symp. Soc., Exp. Biol. 11: 118-131.

Smita shingane (2008) *In vitro* regeneration studies in cotton M. Sc thesis (un pub.) Dr. PDKV, Akola.

Srinivas L., Ganapathi T.R., Suprasanna P, Bapat V.A. 2006: Desiccation and ABA improve conversion of somatic embryo of banana cv. Rasthali (AAB), India J. Biotechnol. 5: 521-526.

Stelly, D.M., D.W. Altman, R.J. Kohel, T.S. Rangan and E. Commiskey, 1989: Cytogenetic abnormalities of cotton somaclones from callus cultures. Genome 32: 762-770.

Steward, F.C., 1958: Growth and development of cultivated cells. III. Interpretations of the growth from free cell to carrot plant. Am. J. Bot. 45:709-713.

Steward, F.C., H.W. Israel, R.L. Mott, H.J. Wilson and A.D. Krikorian, 1975: Observations on growth and morphogenesis in cultured cells of carrot (*Daucus carota* L.) Phil. Trans, Roy. Soc., Landon, 273: 33-53.

*Sun-YuQiang, Zhang – XianLong, Jin-Shuang Xiz, Liang – shao Guang and Nie-Yiehun, 2003: Somatic embryogenesis and plant regeneration in wild cotton (*Gossypium Klotzchianum*), Plant Cell Tissue and Organ Culture 75 (3): 247-253.

Suresh Kumar, M.V., I.S. Katageri and R.R. Soddi, 2002: Callus Induction and regeneration studies in *G. hirsutum*, Cotton Res. Dev., 16 (1): 8-12.

Suresh Kumar M.V., I. S. Karageri, H.M. Vamadeviah, B.M.Khadi and P.M. Salimath, 2003: *In vitro* screening for regeneration in cotton (*Gossypium* spp.), Indian J.Genet. 63(1): 54-58.

Sucheta Tripathy and G.M. Reddy, 2002: *In vitro* Callus induction and plantlet regeneration from Indian Cotton cultivars. Plant Cell Biotechnology and Molecular Biology 3 (3/4): 137-142.

Suprasanna P, C. Rupali, N.S. Desai and V.A. Bapat 2008: Partial desiccation augments plant regeneration from irradiated embryogenic cultures of sugarcane. Plant Cell Tissue and Organ Culture 92:101-105

Thomas, M.R, L.B. Johnson and F.F. White.1995: Selection of interspecific somatic hybrids of medicago by using Agrobacterium transformed tissues. Plant Sci. 69:189-198.

Trolinder, N. L. and J.R. Goodin, 1987: Somatic embryogenesis and plant regeneration in cotton (*Gossypioum hirsutum* L.) Plant Cell Reports,6: 231 –234.

Trolinder, N. L. and J.R. Goodin, 1988a: Somatic embryogenesis in cotton (*Gossypium hirsutum*) i. Effect of source of explants and hormone regime. Plant Cell, Tissue and Organ Culture, 12: 31-42.

Trolinder, L.N. and J.R. Goodin, 1988b: Somatic embryogenesis in cotton (*Gossypium*) ii. Requirements for embryo development and plant regeneration, Plant Cell, Tissue and Organ Culture, 12: 43-53.

Trolinder, N.L. and C. XhiXhian, 1989: Genotypes specificity of the somatic embryogenesis response in cotton. Plant Cell Report 8: 133-138.

Tsukahara M., Hirosawa T. 1992 Simple dehydration treatment promotes plantlelts regeneration of rice (*Oryza sativa* L.) Callus. Plant cell Report 11: 530 – 553.

Umberk, P, G. Johanson and K. Barton.1987: Genetically transformed cotton (*Gossypium hirsutum* L.) Plants. Biotechnology. 5: 235-236.

Van overbeek, J., Conklin, M.E. and Blakslee, A.F. 1941: Factors in coconut milk essential for growth and development of very young Datura embryos. Science 94:350–351.

Verma, S.K., P. Bhatia, S. Bhutani, R.K. Jain and V.K. Chaudhary, 2005: *In vitro* Studies on callus Induction and Plant regeneration in upland cotton varieties and hybrids of North India. J. Indian Soc. Cotton Improve: 97-103.

Vertucci C.W., Farrant J.M. 1995: Acquisition and loss of desiccation tolerance. In Kigal J, Galili G. (eds).Seed development and germination. Marcel Dekker, New York, USA, pp. 237-271.

Voo, K.S., Rugh, C.L. and J.C. Kamalay, 1991: Indirect somatic embryogenesis and plant recovery from cotton (*G.hirsutum* L.) *In vitro* cellular and development biology plant. 27 (3): 117 – 124

Wagiran A., I. Ismail, M. Zain and R. Abdullah 2008: Improvement of plant regeneration from embryogenic suspension cell culture of Japonica rice. Journal of Biological Science 8(3): 570-576.

White, P.R.1939: Potentially unlimited growth of excised plant callus in artificial nutrient medium. Am. J. Bot 26: 59-64.

*Winkler, H. 1908: Besprechung der Arbeit G. Haberland's Kultur versuche mit isolierten pflanzen Zellen. Bot. Z. 60: 262 – 2264.

*Wilkins, T., K. Rajasekaran and D.M. Anderson, 2000: Cotton biotechnology. Crit. Rev. Plant, Sci. 19: 511-550.

*Xue Meifeng, Guo-Yulong, Li-Ming yang and Pei-Yan. 2002: Effect of long-term subculture on embryogenesis capability of cotton callus and somatic variation. South west China J. Agricultural Sciences 15 (4): 19-21.

Yehosud S., Rhudes. D., and J. Janick, 1992: Changes in amino acid composition associated with tolerance to partial desiccation of celery somatic embryos. J. Am. Soc. Horti. Sci. 117:337–341.

Zhang, BaoHong, Liu-Fang and Yao-Chang Bing, 2000: Plant regeneration via somatic embryogenesis in cotton.Plant Cell Tissue and organ Culture 60 (2): 89-94.

*Zhang, Bao Hong, Feng Rong, Liu Fang, Zhou DaYun and Wang Qinglian, 2001: Direct somatic embryogenesis and plant regeneration from cotton (*Gossypium hirsutum* L.) explants. Israel J. plant Sciences. 49 (3): 193-196.

Zhang, BaoHong, Feng Rong, Liu Fang and Wong Qing Lian, 2001: High frequency Somatic embryogenesis and plant regeneration of an elite Chinese Cotton Variety. Bot. Bull. Acad. Sin. 42: 9-6.

*Zhang Han Shuang, Li Jun Lan, Gao Peng and Wang Hai Bo. 1999: Embryogenesis and plant regeneration from somatic cell culture of glandless upland cotton. J. Hebei Agril. University 22(1): 9-12.

* Originals not seen

VITA

1. Name of student : **Zambre Supriya Manohar**
2. Date of Birth : **24th Sep 1985**
3. Name of College : **Post Graduate Institute, Dr. PDKV, Akola, Maharashtra.**
4. Residential address along with phone no. : **C/O Shri. Manohar A. Zambre, Raj colony Keshav nagar, Akola Dist- Akola, Pin- 4440004 Ph. No. 9922392124**

5. Academic qualifications

Sr. No.	Name of Degrees awarded	Year in which obtained	Division / Class	Name of awarding university	Subjects
1.	B.Sc. (Agriculture)	2007	Second	Dr.PDKV, Akola	Agriculture

6. Field of Interest : Research and Development

Place: Akola

Date : 12 **/ 05/ 2009**

Zambre

Signature of Student