

**MORPHOGENESIS AND TISSUE CULTURE STUDIES OF
Gerbera jamesonii BOLUS Ex. Hook F.**

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**FACULTY OF SCIENCE
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MORPHOGENESIS AND TISSUE CULTURE STUDIES OF
Gerbera jamesonii Bolus Ex. Hook F.

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ABSTRACT

In vitro regeneration of *Gerbera jamesonii* Bolus ex. Hook f. was successfully carried out in this study. Petiole and leaf explants from 8-week-old aseptic seedlings were used as source of explants. Different response was observed when different explants were cultured on the culture media. MS (Murashige and Skoog, 1962) supplemented with 2.0 mg/l BAP and 0.5 mg/l NAA was identified as the optimum medium for shoot regeneration, while petiole explant was identified as the most responsive explant in this study. Petiole explants regenerated shoots (94.3 ± 2.5 %) and produced 9.3 ± 0.6 shoots per explant. MS basal medium was the optimum medium for plantlet root induction. Various concentrations of auxin such as NAA, IAA, IBA and 2,4-D at a range of 0.1-2.0 mg/l and cytokinin such as BAP, Kinetin, Zeatin and 2iP at a range of 0.1-3.0 mg/l were used in order to achieve *in vitro* regeneration of *G. jamesonii*.

Callus was also induced from *G. jamesonii* petiole and leaf explants. Optimum callus induction was obtained when petiole explant was cultured on MS medium supplemented with 2.0 mg/l 2,4-D and 1.0 mg/l BAP. Green compact callus were induced. Through screening of secondary metabolites in *Gerbera* callus, flavonoid, terpenoid and conjugated chain compounds were found in *Gerbera* callus.

Studies on indirect somatic embryogenesis of *G. jamesonii* were carried out using leaf explant. Induction of embryogenic callus was obtained when petiole explants were cultured on MS media supplemented with 0.01-2.0 mg/l 2,4-D. White friable callus was induced. Embryogenic callus was distinguished from non-embryogenic callus through

double staining method. Embryogenic callus obtained were transferred to MS liquid medium fortified with 0.1-2.0 mg/l 2,4-D added with 0.1 or 1.0 mg/l NAA. Somatic embryos produced from embryogenic callus cultured in liquid medium were then transferred to MS medium supplemented with 1.0 mg/l BAP and 0.1 mg/l NAA added with 50 mM L-Proline. A total of 29.8 ± 1.2 embryos per explant were obtained.

Synthetic seeds of *G. jamesonii* were successfully produced when micro shoots, globular and cotyledonary phase somatic embryos were encapsulated with encapsulation matrix composed of 3.0% sodium alginate dissolved in Ca-free MS liquid medium supplemented with 2.0 mg/l BAP and 0.5 mg/l NAA. Germination rates obtained from encapsulated micro shoots was $74.5 \pm 2.6\%$, $34.8 \pm 1.2\%$ for encapsulated globular somatic embryos and $54.2 \pm 1.3\%$ for encapsulated cotyledonary phase somatic embryos. Synthetic seeds from encapsulated micro shoots could be stored at 4 ± 1 °C for 180 days while synthetic seeds from encapsulated globular and cotyledonary phase somatic embryos could be stored at 4 ± 1 °C up to 90 days only before being germinated.

Generally, *in vitro* regeneration from petiole explants were reduced when explants were exposed to gamma irradiation (10-60 Gy). As the irradiation dose increased, *in vitro* shoots regeneration was declined and abnormal shoots were formed. *In vitro* plantlets exposed to gamma irradiation also showed height reduction as the irradiation dose was increased. Effects of gamma irradiation were also observed on callus tissues. Growth of *G. jamesonii* callus tissues was also reduced with the increase of the irradiation dose. Reduction of chlorophyll content in callus tissues was observed when *Gerbera* callus

were exposed to gamma irradiation. Soluble protein content in callus tissues were also reduced with the increase of irradiation dose.

Gerbera jamesonii plantlets obtained from tissue culture system through direct regeneration, somatic embryogenesis, synthetic seeds and also irradiated plantlets were successfully transferred and acclimatized to the new environment. Garden soil and vermiculite were used as sowing media. Growth and development of plantlets were optimum when plantlets obtained from regeneration of petiole explants were acclimatized in garden soil (combination of black soil and red soil at 2:1) with $86.0 \pm 0.9\%$ survival. *Ex-vitro* flowering was achieved after 6 months. *Gerbera jamesonii* plants obtained from *in vitro* regeneration showed similar morphological characters to the mother plant.

ABSTRAK

Regenerasi lengkap bagi tumbuhan *Gerbera jamesonii* Bolus ex. Hook f. daripada sistem kultur tisu telah berjaya diperoleh. Eksplan petiol dan daun daripada anak benih aseptik yang berumur 8 minggu telah digunakan untuk tujuan ini. Respons yang berbeza telah diperoleh apabila eksplan yang berlainan dikultur di atas media kultur. Medium MS (Murashige and Skoog, 1962) ditambah dengan kombinasi hormon 2.0 mg/l BAP dan 0.5 mg/l NAA telah dikenalpasti sebagai medium optima bagi regenerasi pucuk manakala eksplan petiol pula telah dikenalpasti sebagai eskplan yang terbaik dalam kajian ini. Eksplan petiol telah berjaya menghasilkan pucuk *in vitro* ($94.3 \pm 2.5\%$) dengan 9.3 ± 0.6 pucuk dihasilkan bagi setiap eksplan. Medium MS tanpa hormon merupakan medium yang optima untuk penginduksian akar. Pelbagai kombinasi auksin seperti NAA, IAA, IBA dan 2,4-D dalam julat antara 0.1-2.0 mg/l dan sitokinin seperti BAP, Kinetin, Zeatin dan 2iP pada julat 0.1-3.0 mg/l telah digunakan untuk tujuan regenerasi lengkap tumbuhan *G. jamesonii* secara *in vitro*.

Induksi kalus juga telah dijalankan ke atas eksplan petiol dan daun tumbuhan *G. jamesonii*. Penginduksian kalus optima telah diperoleh apabila eksplan petiol dikultur di atas media MS dengan 2.0 mg/l 2,4-D dan 1.0 mg/l BAP. Kalus yang dihasilkan bewarna hijau dan mempunyai struktur yang padat. Kajian penskrinan bahan metabolit sekunder ke atas kalus yang dihasilkan mendapati kalus daripada tumbuhan *Gerbera* mengandungi flavonoid, terpenoid dan juga sebatian rantai berkonjugat.

Kajian embriogenesis somatik secara tidak langsung telah dijalankan ke atas eksplan daun. Pengaruh kalus embriogenik telah diperoleh apabila eksplan petiol dikultur di atas medium MS ditambah dengan 0.01-2.0 mg/l 2,4-D. Kalus yang dihasilkan bewarna keputihan yang mempunyai struktur yang lembut dan rapuh. Kalus embriogenik telah dibezakan dengan kalus bukan embriogenik dengan menggunakan kaedah pewarnaan berganda. Kalus embriogenik yang dihasilkan kemudian dipindahkan ke dalam media cecair MS yang mengandungi 0.1-2.0 mg/l 2,4-D yang ditambah dengan 0.1 atau 1.0 mg/l NAA. Embrio somatik dihasilkan apabila kalus embriogenik daripada kultur ampaian dipindahkan ke atas media MS dengan 1.0 mg/l BAP dan 0.1 mg/l NAA dan ditambah dengan 50 mM L-Proline dengan penghasilan embrio sebanyak 29.8 ± 1.2 embrio bagi setiap eksplan yang telah digunakan.

Biji benih tiruan bagi tumbuhan *G. jamesonii* telah berjaya diperoleh apabila pucuk mikro, embrio somatik peringkat globul dan kotiledon disalut menggunakan matriks pengkapsulan yang dihasilkan daripada 3.0% larutan sodium alginat yang dilarutkan di dalam media cecair MS tanpa kalsium dan ditambah dengan 2.0 mg/l BAP dan 0.5 mg/l NAA. Peratus percambahan biji benih tiruan daripada eksplan pucuk mikro adalah $74.5 \pm 2.6\%$, $34.8 \pm 1.2\%$ bagi embrio somatik peringkat globul dan $54.2 \pm 1.3\%$ bagi embrio somatik peringkat kotiledon. Biji benih tiruan yang dihasilkan daripada pucuk mikro dapat disimpan pada suhu 4 ± 1 °C sehingga 180 hari manakala biji benih tiruan yang dihasilkan daripada pengkapsulan eksplan somatik embrio peringkat globul dan kotiledon hanya dapat disimpan pada suhu 4 ± 1 °C sehingga 90 hari sebelum dicambahkan.

Penghasilan pucuk *in vitro* daripada eksplan petiol telah didapati merosot apabila eksplan didedahkan kepada sinaran gamma (10-60 Gy). Semakin tinggi dos pendedahan kepada sinaran gamma, penghasilan pucuk *in vitro* semakin berkurangan dan pucuk abnormal mula terhasil. Plantlet *in vitro* yang didedahkan kepada sinaran gamma juga mengalami kesan radiasi di mana ketinggian plantlet semakin berkurangan apabila kadar radiasi bertambah. Kesan sinaran radiasi gamma juga dapat diperhatikan ke atas tisu kalus. Kadar perkembangan tisu kalus tumbuhan *Gerbera* semakin berkurangan dengan pertambahan kadar sinaran radiasi yang didedahkan kepada tisu kalus tersebut. Analisa ke atas kandungan klorofil di dalam tisu kalus mendapati bahawa kandungan klorofil semakin berkurangan dengan pertambahan dos sinaran gamma yang didedahkan. Kandungan protein terlarut di dalam tisu kalus juga berkurangan dengan pertambahan dos sinaran gamma yang didedahkan.

Plantlet tumbuhan *G. jamesonii* yang berjaya dihasilkan daripada sistem kultur tisu samada daripada regenerasi secara langsung, embriogenesis somatik, biji benih tiruan dan juga plantlet yang dihasilkan melalui regenerasi *in vitro* yang telah didedahkan kepada sinaran radiasi gamma telah berjaya dipindahkan ke persekitaran luar dan menjalankan proses aklimatisasi. Tanah kebun dan juga vermiculite telah digunakan sebagai substrat pertumbuhan. Pertumbuhan dan perkembangan plantlet yang diaklimatisasi didapati optima apabila plantlet yang dihasilkan daripada regenerasi eksplan petiol dipindahkan ke tanah kebun (campuran tanah hitam dan tanah merah pada nisbah 2:1) dengan peratus keterushidupan sebanyak $86.0 \pm 0.9\%$. Pembungaan *ex-vitro* telah dapat diperhatikan selepas 6 bulan. Perbandingan dari segi morfologi mendapati bahawa tumbuhan

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TABLE OF CONTENTS

Title	Page
ABSTRACT	i
ABSTRAK	iv
ACKNOWLEDGEMENT	viii
TABLE OF CONTENTS	ix
LIST OF TABLES	xvii
LIST OF FIGURES	xxi
LIST OF ABBREVIATIONS	xxvi
CHAPTER 1 INTRODUCTION	1
1.1 GENERAL INTRODUCTION	1
1.2 INTRODUCTION TO THE FAMILY ASTERACEAE (COMPOSITAE)	31
1.2.1 Genus <i>Gerbera</i> (African Daisy)	33
1.3 RESEARCH OBJECTIVES	37
CHAPTER 2 <i>IN VITRO</i> REGENERATION OF <i>Gerbera jamesonii</i> Bolus Ex. Hook f.	44
2.1 EXPERIMENTAL AIMS	44
2.2 MATERIALS AND METHODS	48
2.2.1 Source of Explants	48
2.2.2 Preparation of Aseptic Seedlings	48
2.2.3 Type of Explants	48
2.2.4 Sterilization of Explants	49
2.2.5 Preparation of Culture Media	49

2.2.5.1	Preparation of MS Stock Solution	50
2.2.5.2	Preparation of Basic Medium, MS (1962)	50
2.2.5.3	Preparation of Culture Media with Hormones	51
2.2.6	Culture Conditions	51
2.2.7	Subculture	52
2.2.8	Plant Regeneration <i>In Vitro</i>	52
2.2.8.1	Identification of the Best Polarity of Explants for Shoot Regeneration	52
2.2.8.2	Identification of Shoot Regeneration Media	53
2.2.8.2(a)	Identification of suitable BAP and NAA Combination for Shoot Regeneration	53
2.2.8.2(b)	Identification of Other Suitable Cytokinin and Auxin for Shoot Regeneration	55
2.2.8.3	Identification of Root Induction Media	57
2.2.8.4	Identification of Optimum Sucrose Concentration for Regeneration of Shoot	57
2.2.8.5	Identification of Optimum pH Media on Regeneration of Shoots	58
2.2.8.6	Effect of Liquid and Solid Media on Formation of Adventitious Shoot	58
2.2.8.7	Effect of Coconut Water on Shoot Regeneration	59
2.2.9	Microscopic Studies (Scanning Electron Microscopy-SEM)	59
2.2.10	Data analysis	60
2.3	RESULTS	61
2.3.1	Identification of the Best Polarity of Explants for Shoot Regeneration	61
2.3.2	Identification of Shoot Regeneration Media	64
2.3.3	Identification of Root Induction Media	75
2.3.4	Effect of Liquid and Solid Media on Formation of Adventitious shoot	77

2.3.5 Identification of Optimum Sucrose Concentrations for Shoot Regeneration	79
2.3.6 Identification of Optimum pH Media for Shoot Regeneration	81
2.3.7 Effect of Coconut Water on Regeneration of Shoot	83
2.3.8 Microscopic Studies (Scanning Electron Microscopy-SEM)	85
2.3.9 Data Analysis	85
2.4 SUMMARY OF RESULTS	90
CHAPTER 3 CALLUS INDUCTION FROM VARIOUS EXPLANTS OF <i>Gerbera jamesonii</i> Bolus Ex. Hook f.	94
3.1 EXPERIMENTAL AIMS	92
3.2 MATERIALS AND METHODS	94
3.2.1 Seed Sterilization	94
3.2.2 Callus Induction	94
3.2.3 Data Analysis	96
3.2.4 Studies on Secondary Metabolites in Callus	96
3.3 RESULTS	98
3.3.1 Callus Induction	98
3.3.2 Screening of Secondary Metabolites	104
3.4 SUMMARY OF RESULTS	106

CHAPTER 4	SOMATIC EMBRYOGENESIS INDUCTION IN	107
	<i>Gerbera jamesonii</i> Bolus Ex. Hook f.	
4.1	EXPERIMENTAL AIMS	107
4.2	MATERIALS AND METHODS	109
4.2.1	Preparation of Explants	109
4.2.2	Preparation of Culture Medium and Callus Induction	109
4.2.3	Identification of Embryogenic Callus	110
4.2.4	Embryogenic Callus Initiation and Establishment of Cell Suspension Culture	110
4.2.5	Induction of Somatic Embryos	111
4.2.6	Development of Plantlets from Somatic Embryos	111
4.2.7	Microscopic Studies	111
4.2.8	Data Analysis	112
4.3	RESULTS	113
4.3.1	Induction and Identification of Embryogenic Callus	113
4.3.2	Induction and Development of Somatic Embryos	120
4.4	SUMMARY OF RESULTS	130
CHAPTER 5	PRODUCTION OF SYNTHETIC SEEDS OF	131
	<i>Gerbera jamesonii</i> Bolus Ex. Hook f.	
5.1	EXPERIMENTAL AIMS	131
5.2	MATERIALS AND METHODS	134
5.2.1	Production of Micro Shoots	134
5.2.2	Preparation of MS Basal Medium	134
5.2.3	Preparation of Sodium Alginate Solution	134

5.2.4 Preparation of Calcium chloride Dehydrate Solution	135
5.2.5 Encapsulation Techniques and Formation of Synthetic Seeds	135
5.2.5.1 Sodium Alginate Solution and Calcium Chloride Dehydrate Solution in Different Concentrations	136
5.2.5.2 Preparation of Encapsulation Matrix in Different Solution	137
5.2.6 Germination of Synthetic Seeds on Various Sowing Media	137
5.2.7 Low Temperature Storage	138
5.2.8 Data Analysis	138
5.3 RESULTS	139
5.3.1 The Effect of Different Concentrations of Sodium Alginate Solution and Calcium Chloride Dehydrate Solution on Bead Formation	139
5.3.2 Germination of <i>Gerbera</i> Synthetic Seeds on Different Germinating Media	139
5.3.3 Encapsulation Matrix in Different Solution	145
5.3.4 Effect of Different Types of Sowing Media on Germination of Synthetic Seed	145
5.3.5 Low Temperature Storage	149
5.4 SUMMARY OF RESULTS	151

CHAPTER 6	PLANT REGENERATION FROM SYNTHETIC SEEDS OF <i>Gerbera jamesonii</i> Bolus Ex. Hook f.	152
6.1	EXPERIMENTAL AIMS	152
6.2	MATERIALS AND METHODS	154
6.2.1	Preparation of Explants	154
6.2.2	Formation of Synthetic Seeds	154
6.2.3	Plant Regeneration from Synthetic Seeds	154
6.2.3.1	Germination media	155
6.2.3.2	Effects of Different Sucrose Concentrations in Culture Medium	156
6.2.3.3	Effects of Hormone in Encapsulaiton Matrix	156
6.2.4	Storage of Synthetic Seeds	157
6.2.5	Data Analysis	157
6.3	RESULTS	158
6.3.1	Germination of Synthetic Seeds	158
6.3.2	Effects of Storage Period on Germination of Synthetic Seeds	175
6.4	SUMMARY OF RESULTS	178
CHAPTER 7	EFFECTS OF IRRADIATION ON CULTURES OF <i>Gerbera jamesonii</i> Bolus Ex. Hook f.	180
7.1	EXPERIMENTAL AIMS	180
7.2	MATERIALS AND METHODS	183
7.2.1	Source of Explants	183
7.2.2	Source of Gamma Radiation	183
7.2.3	Gamma Radiation Dose	183

7.2.4	Effect of Gamma Radiation on Shoot Regeneration	184
7.2.5	Effect of Gamma Radiation on <i>In Vitro</i> Propagated Shoots	185
7.2.6	Effect of Gamma Radiation on Callus Growth	185
7.2.7	Chlorophyll Extraction and Determination	186
7.2.7.1	Calculation of Total Chlorophyll Content In Irradiated Callus Tissues	187
7.2.8	Soluble Protein Extraction	188
7.2.8.1	Soluble Protein Analysis	188
7.2.9	Data Analysis	189
7.3	RESULTS	190
7.3.1	Effects of Gamma Irradiation on Regeneration of Shoots from Petiole Explant	190
7.3.2	Effects of Gamma Irradiation on Regeneration of <i>In Vitro</i> Propagated Shoots	194
7.3.3	Effects of Gamma Irradiation on Callus Tissues	197
7.3.4	Chlorophyll a, Chlorophyll b and Total Chlorophyll Content of Irradiated Callus	201
7.3.5	Soluble Protein Content in Irradiated Callus	204
7.4	SUMMARY OF RESULTS	208
CHAPTER 8	ACCLIMATIZATION OF PLANTLETS OF <i>Gerbera jamesonii</i> Bolus Ex. Hook f.	209
8.1	EXPERIMENTAL AIMS	209
8.2	MATERIALS AND METHODS	211
8.2.1	Source of <i>In Vitro</i> Plantlets	211
8.2.2	Development and Growth of <i>In Vitro</i> Plantlets	211

8.2.2.1	Transplantation of <i>In Vitro</i> Plantlets to Various Media or Substrates and Acclimatization Process	211
8.2.3	Transferring of Plantlets to the Green House and Acclimatization of <i>Gerbera</i> Plantlets Obtained from Various Culture Protocols	212
8.2.4	The Effect of Different Environmental Factors on Acclimatization of <i>Gerbera jamesonii</i>	213
8.2.5	Measurement of Chlorophyll Content	214
8.2.6	Macromorphology Studies	214
8.2.7	Data Analysis	214
8.3	RESULTS	215
8.3.1	Transferring of <i>In Vitro</i> Plantlets to Various Media or Substrates and Acclimatization Process	215
8.3.2	Transferring of Plantlets to the Green House and Acclimatization of <i>Gerbera</i> Plantlets Derived from Various Treatments	218
8.3.3	The Effect of different Environmental Conditions on Acclimatization	219
8.3.4	Measurement of Chlorophyll Content	224
8.3.5	Macromorphology Studies of <i>Gerbera jamesonii</i>	226
8.4	SUMMARY OF RESULTS	231
CHAPTER 9	DISCUSSION	233
CHAPTER 10	CONCLUSION	290
REFERENCES		295
APPENDIX		334

. LIST OF TABLES

Table 2.1	Responses of different polarity of explants cultured on MS media supplemented with 1.0 mg/l BAP and 1.0 mg/l NAA maintained at 25 ± 1 °C and 16 hours light and 8 hours dark for 8 weeks.	63
Table 2.2	The effect of different concentrations and combinations of BAP and NAA on leaf and petiole explants cultured on MS media at 25 ± 1 °C with 16 hours light and 8 hours dark.	67
Table 2.3	The effect of different combinations of auxin (2, 4-D, IBA, IAA and NAA) at the concentration of 0.5 mg/l and Cytokinin (BAP, 2iP, Kinetin and Zeatin) at the concentration of 2.0 mg/l on petiole explant cultured on MS media at 25 ± 1 °C with 16 hours light and 8 hours dark.	71
Table 2.4	Development of roots from <i>in vitro</i> shoots in rooting media after 4 Weeks. Cultures were maintained at 25 ± 1 °C with 16 hours light and 8 hours dark.	76
Table 2.5	Multiplication of shoots after 4 weeks being cultured in solid and liquid media. Cultures were maintained at 25 ± 1 °C with 16 hours light and 8 hours dark.	78
Table 2.6	The effect of different sucrose concentration in regeneration of shoots. Cultures were maintained at 25 ± 1 °C with 16 hours light and 8 hours dark.	80
Table 2.7	The effect of different pH in regeneration of shoots. Cultures were maintained at 25 ± 1 °C with 16 hours light and 8 hours dark.	82
Table 2.8	The effect of coconut water for regeneration of shoots from petiole explant. Cultures were maintained at 25 ± 1 °C with 16 hours light and 8 hours dark.	84

Table 3.1	Callus induction from leaf explants of <i>Gerbera jamesonii</i> Bolus ex. Hook f.	100
Table 3.2	Callus induction from petiole explants of <i>Gerbera jamesonii</i> Bolus ex. Hook f.	101
Table 3.3	Thin layer chromatography (TLC) on methanol extract of callus of <i>Gerbera jamesonii</i> Bolus ex. Hook f.	105
Table 4.1	Induction of callus from leaf explants cultured on MS medium supplemented with 2, 4-D after 8 weeks of culture.	115
Table 4.2	Induction of callus from leaf explants cultured on MS medium supplemented with TDZ after 8 weeks of culture.	116
Table 4.3	Induction of callus from leaves explants cultured on MS medium supplemented with BAP and 2,4-D after 8 weeks of culture.	117
Table 4.4	Effects of BAP , NAA and L-Proline on formation of somatic embryos of <i>Gerbera jamesonii</i> Bolus ex. Hook f.	122
Table 4.5	Composition of culture medium and growth condition for <i>Gerbera jamesonii</i> somatic embryo induction	123
Table 5.1	Effect of different concentrations of sodium alginate ($\text{NaC}_6\text{H}_7\text{O}_6$) and calcium chloride ($\text{CaCl}_2\cdot\text{H}_2\text{O}$) on bead formation.	141
Table 5.2	Effect of different concentrations of Sodium Alginate ($\text{NaC}_6\text{H}_7\text{O}_6$) and Calcium Chloride ($\text{CaCl}_2\cdot\text{H}_2\text{O}$) solution on germination of <i>Gerbera jamesonii</i> Synthetic Seed on solid and liquid Media.	142
Table 5.3	Growth response of micro shoots of <i>Gerbera jamesonii</i> encapsulated in different encapsulation matrix.	146
Table 5.4	Effect of different sowing media on germination rate of synthetic seeds of <i>Gerbera jamesonii</i> .	147
Table 5.5	Effect of storage period (days) at 4 ± 1 °C on germination of synthetic seeds of <i>Gerbera</i> on germination MS basal medium.	150

Table 6.1	<i>In vitro</i> germination of synthetic seeds of <i>Gerbera jamesonii</i> (micro shoots, globular phase, cotyledonary phase somatic embryos). Results were observed based on germination period (days).	161
Table 6.2	<i>In vitro</i> germination of synthetic seeds of <i>Gerbera jamesonii</i> (micro shoots, globular phase, cotyledonary phase somatic embryos). Results were observed based on germination rate (%).	162
Table 6.3	Germination of synthetic seeds of <i>Gerbera jamesonii</i> (micro shoots, globular phase, cotyledonary phase somatic embryos). Results were observed based on survival rate (%) after 8 weeks of germination.	165
Table 6.4	Effect of sucrose at different concentrations on germination rate of Synthetic Seeds of <i>Gerbera jamesonii</i> .	170
Table 6.5	Growth response of micro shoots and somatic embryo of <i>Gerbera</i> encapsulated in different encapsulation matrix. Results were observed based on germination rate (%) after 10 days of germination.	171
Table 6.6	Growth response of micro shoots and somatic embryos of <i>Gerbera jamesonii</i> encapsulated in different encapsulation matrix. Results were observed based on survival rates (%) after 8 weeks of germination.	172
Table 6.7	Effect of storage period (days) at 4 ± 1 °C on germination of synthetic seeds of <i>Gerbera jamesonii</i> , germinated on MS basal medium.	177

Table 7.1	The effects of gamma irradiation on regeneration of shoots from petiole explants of <i>Gerbera jamesonii</i> . Irradiated explants were cultured on MS medium supplemented with 2.0 mg/l BAP and 0.5 mg/l NAA. Cultures were incubated in the culture room at 25 ±1 °C with 16 hours light and 8 hours dark for 8 weeks.	192
Table 7.2	The effects of gamma irradiation on regeneration of <i>in vitro</i> propagated shoots of <i>Gerbera jamesonii</i> . Irradiated shoots were cultured on MS medium supplemented with 2.0 mg/l BAP and 0.5 mg/l NAA. Cultures were incubated in the culture room at 25 ±1 °C with 16 hours light and 8 hours dark for 8 weeks.	195
Table 7.3	The effects of gamma irradiation on callus of <i>Gerbera jamesonii</i> . Irradiated callus were cultured on MS medium supplemented with 2.0 mg/l BAP and 0.5 mg/l NAA. Cultures were incubated in the culture room at 25 ±1 °C with 16 hours light and 8 hours dark for 8 weeks.	199
Table 7.4	Chlorophyll a, Chlorophyll b and Total Chlorophyll Content of Irradiated Callus of <i>Gerbera jamesonii</i> at different doses of Gamma irradiation.	202
Table 7.5	Soluble Protein Content (µg of fresh weight) in irradiated callus of <i>Gerbera jamesonii</i> at different doses of Gamma irradiation.	205
Table 8.1	Responses showed by <i>in vitro</i> <i>Gerbera</i> plantlets after being acclimatized in various sowing media. Results obtained after 4 weeks plantlets being acclimatized.	217
Table 8.2	Responses showed by <i>in vitro</i> <i>Gerbera</i> plantlets obtained from various sources after being acclimatized in garden soil. Results obtained after 12 weeks plantlets being acclimatized	220
Table 8.3	Comparison of macromorphological characters of <i>in vitro</i> plantlets (control), intact plants, and irradiated plantlets at 20 Gy, 30 Gy and 40 Gy after 6 months being acclimatized on garden soil.	228

LIST OF FIGURES

Figure 1.1	Nine-month-old intact plant of <i>Gerbera jamesonii</i> Bolus ex. Hook f. with flowers.	42
Figure 1.2	Intact flower of <i>Gerbera jamesonii</i> Bolus ex. Hook. f.	42
Figure 1.3	Nine-month-old intact plant of <i>Gerbera jamesonii</i> Bolus ex. Hook f.	43
Figure 2.1	Regeneration of shoots from petiole explants cultured on MS medium supplemented with 2.0 mg/l BAP and 0.5 mg/l NAA	72
Figure 2.2	Regeneration of shoots from petiole explants cultured on MS medium supplemented with 2.0 mg/l Zeatin and 0.5 mg/l IBA.	72
Figure 2.3	Development of roots from leaf explant cultured on MS medium supplemented with 0.1 mg/l BAP and 2.0 mg/l NAA.	73
Figure 2.4	Roots formed from leaf explants cultured on MS medium fortified with 2.0 mg/l NAA.	73
Figure 2.5	<i>In vitro</i> plantlet obtained from regeneration of petiole explant cultured on MS medium supplemented with 2.0 mg/l BAP + 0.5 mg/l NAA. Plantlet was transferred to MS basal media for root elongation.	74
Figure 2.6 (a)	SEM micrograph showing abaxial surface of <i>in vitro</i> leaf of <i>Gerbera jamesonii</i> . Stoma were clearly seen on the leaf.	86
Figure 2.6 (b)	SEM micrograph showing adaxial surface of <i>in vitro</i> leaf of <i>Gerbera jamesonii</i> . Very few stoma were seen on the leaf.	86
Figure 2.6 (c)	SEM micrograph showing abaxial surface of <i>in vivo</i> (intact) leaf of <i>Gerbera jamesonii</i> . Stoma were seen on the leaf.	87
Figure 2.6 (d)	SEM micrograph showing adaxial surface of <i>in vivo</i> (intact) leaf of <i>Gerbera jamesonii</i> . Very few stoma were found on the leaf.	87
Figure 2.6 (e)	SEM micrograph showing stomata on <i>in vitro</i> leaf of <i>Gerbera jamesonii</i> .	88

Figure 2.6 (f)	SEM micrograph showing stomata on <i>in vivo</i> (intact) leaf of <i>Gerbera jamesonii</i> .	88
Figure 2.6 (g)	SEM micrograph showing trichomes on <i>in vitro</i> leaf of <i>Gerbera jamesonii</i> .	89
Figure 2.6 (h)	SEM micrograph showing trichomes on <i>in vivo</i> (intact) leaf of <i>Gerbera Jamesonii</i> .	89
Figure 3.1	Callus derived from leaf explant cultured on MS medium supplemented with 2.0 mg/l 2, 4-D and 1.0 mg/l BAP.	102
Figure 3.2	Callus derived from leaf explants cultured on MS medium supplemented with 1.0 mg/l 2, 4-D and 2.0 mg/l BAP.	102
Figure 3.3	Callus derived from leaf explant cultured on MS medium supplemented with 0.5 mg/l 2, 4-D and 2.0 mg/l BAP.	103
Figure 3.4	Callus derived from petiole explant cultured on MS medium supplemented with 2.0 mg/l 2, 4-D and 1.0 mg/l BAP.	103
Figure 4.1 (a)	Embryogenic callus cells stained red (acetocarmine).	118
Figure 4.1 (b)	Non-embryogenic callus cells stained blue (Evan's Blue).	118
Figure 4.2 (a)	Cross section of embryogenic callus observed under Scanning Electron Microscope. Embryogenic callus with thicker cell wall and friable structure.	119
Figure 4.2 (b)	Cross section of non-embryogenic callus observed under Scanning Electron Microscope. Non-embryogenic cell wall shows compact and thin cell wall.	119
Figure 4.3	Somatic embryogenesis of <i>Gerbera jamesonii</i> from suspension culture.	124
Figure 4.4	Globular and heart shaped stages of somatic embryos formed on embryo induction medium.	124
Figure 4.5 (a)	Globular- shaped phase somatic embryo observed under microphotography microscope.	125

Figure 4.5 (b)	Heart-shaped phase somatic embryo observed under microphotography microscope.	125
Figure 4.5 (c)	Torpedo-shaped somatic embryo observed under microphotography microscope.	126
Figure 4.5 (d)	Cotyledonary phase somatic embryo of <i>Gerbera jamesonii</i> .	126
Figure 4.6 (a)	Globular-shaped phase somatic embryo observed under Scanning Electron Microscope.	127
Figure 4.6 (b)	Heart-shaped phase somatic embryo observed under Scanning Electron Microscope.	127
Figure 4.6 (c)	Torpedo-shaped phase somatic embryo observed under Scanning Electron Microscope.	128
Figure 4.6 (d)	Cotyledonary phase somatic embryo observed under Scanning Electron Microscope.	128
Figure 4.7	Micro shoots developed from somatic embryo of <i>Gerbera jamesonii</i> .	129
Figure 5.1	Micro shoots of <i>Gerbera jamesonii</i> cultured on MS medium supplemented with 3.0 mg/l BAP.	143
Figure 5.2	Ideal beads formed when encapsulation matrix was composed of Ca-free MS basal medium added with 3.0% sodium alginate and 3.0% sucrose and soaked in 100 mM CaCl ₂ .H ₂ O.	143
Figure 5.3	Encapsulated micro shoots of <i>Gerbera jamesonii</i> .	144
Figure 5.4	Plantlet obtained from germination of synthetic seed of <i>Gerbera jamesonii</i> .	148
Figure 6.1	Synthetic seeds of <i>Gerbera jamesonii</i> obtained from the encapsulation of micro shoots.	163
Figure 6.2	Synthetic seeds of <i>Gerbera jamesonii</i> obtained from the encapsulation of cotyledonary phase somatic embryo.	163
Figure 6.3	Germination of synthetic seed of <i>Gerbera jamesonii</i> from encapsulated micro shoots.	173

Figure 6.4	Plantlet obtained from germination of <i>Gerbera</i> synthetic seed derived from encapsulation of micro shoots.	173
Figure 6.5	Plantlet formed from germination of <i>Gerbera jamesonii</i> synthetic seed derived from encapsulation of cotyledonary phase somatic embryos.	174
Figure 6.6	Three-month-old plantlet obtained from germination of <i>Gerbera jamesonii</i> synthetic seed being acclimatized in the green house.	174
Figure 7.1 (a)	Regeneration of shoots from irradiated (30 Gy) petiole explants of <i>Gerbera jamesoni</i> cultured on non-irradiated culture medium.	193
Figure 7.1 (b)	Regeneration of shoots from irradiated (40 Gy) petiole explants of <i>Gerbera jamesoni</i> cultured on non-irradiated culture medium.	193
Figure 7.1(c)	Regeneration of shoots from irradiated (60 Gy) petiole explants of <i>Gerbera jamesoni</i> cultured on non-irradiated culture medium.	193
Figure 7.2 (a)	Three-month-old non-irradiated plantlets acclimatized and transferred to the green house.	196
Figure 7.2(b)	Three-month-old irradiated plantlets at 10 Gy acclimatized and transferred to the green house.	196
Figure 7.2(c)	Three-month-old irradiated plantlets at 20 Gy acclimatized and transferred to the green house.	196
Figure 7.2(d)	Three-month-old irradiated plantlets at 30 Gy acclimatized and transferred to the green house.	196
Figure 7.3(a)	Irradiated callus at 20 Gy cultured on non-irradiated culture medium.	200
Figure 7.3(b)	Irradiated callus at 30 Gy cultured on non-irradiated culture medium.	200
Figure 7.3(c)	Irradiated callus at 40 Gy cultured on non-irradiated culture medium.	200

Figure 7.4	Chlorophyll a, Chlorophyll b and Total Chlorophyll Content of Irradiated Callus of <i>Gerbera jamesonii</i> incubated at $25 \pm 1^{\circ}$ C with 16 hours light and 8 hours dark.	203
Figure 7.5	Protein Standard Curve.	206
Figure 7.6	Soluble Protein Level (μ g of fresh weight) in Irradiated Callus of <i>Gerbera jamesonii</i> incubated at $25 \pm 1^{\circ}$ C and 16 hours light and 8 hours dark.	207
Figure 8.1	The effect of different environmental conditions on <i>Gerbera jamesonii</i> during acclimatization.	222
Figure 8.2	Two-month-old <i>in vitro</i> plantlets growing on garden soil and covered with plastic for acclimatization process.	223
Figure 8.3	Three-month-old <i>Gerbera jamesonii</i> plantlets ready to be acclimatized in the green house.	223
Figure 8.4:	Comparison of chlorophyll content between intact plants, <i>in vitro</i> plantlets, 2-month old, 5-month-old and 12-month-old acclimatized <i>in vitro</i> plantlets.	225
Figure 8.5	Flower bud produced after 23 weeks <i>in vitro</i> plantlet being acclimatized in the green house.	229
Figure 8.6	Six-month-old <i>Gerbera</i> plantlet obtained from <i>in vitro</i> system, acclimatized in the green house with flower bud.	229
Figure 8.7	Six-month-old flowering <i>Gerbera</i> plant obtained from regeneration of petiole explant	230
Figure 8.8	Flower of <i>Gerbera jamesonii</i> from <i>in vitro</i> plantlet after being acclimatized for 6 months in the green house.	230

LIST OF ABBREVIATIONS

BAP	Benzylaminopurine
$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$	Calcium chloride dehydrate
2,4-D	2,4- Dichlorophenoxyacetic acid
HCL	Hydrochloric acid
IAA	Indole-3-acetic acid
IBA	Indolebutyric acid
2-iP	2-isopentenylaminopurine
Kinetin	6-furfurylaminopurine
kPa	Kilo Pasca
mg/l	Milligram per liter
min	minute
MS	Murashige and Skoog
MgCO_3	Magnesium carbonate
NAA	Naphthalene acetic acid
NaOH	Sodium hydroxide
$\text{NaC}_6\text{H}_7\text{O}_6$	Sodium alginate
Rpm	Rotation per minute
SEM	Scanning electron microscope
TDZ	Thidiazuron
Tween 20	Polyoxyethylene sorbitan monolaurate
v/v	Volume per volume
w/v	Weight per volume