

Sudan University of Science and Technology

College of Higher Studies

**Determination of Appropriate Media for *In. Vitro* Culture of
Date Palm (*Phoenix dactylifera* L.)**

**تحديد أوساط غذائية مثلي لزراعة انسجة نخيل التمر (*Phoenix*
dactylifera L.) في**

الانابيب

***Thesis :Submitted in partial fulfillment of the requirements for
the degree of Doctor of
Philosophy in Forestry***

By

Khalid Hamza Elhag Ibrahim AboBaker

B.Sc. Al-Azhar University

1985

M.Sc. University of Khartoum

1997

Supervisor, Dr. Prof. Abdulghaffar El-Hag Said

Sudan (2009)

DEDICATION

***This thesis is dedicated to all my Family, Teachers, Friends and
to my Parents Soul***

ACKNOWLEDGEMENT

I would like to give my special gratitude and deep appreciation and recognition to my supervisor, Dr. prof Abdulghaffar El-Hag Said, who had provided endless support patience, guidance and who offered me acquaintanceship, criticism and highlghtings. Special gratitude and deep appreciation is extended to Dr. Ahmed Mohammed Al-Doma first co supervisor and Dr.Tag El-Seer Ibrahim Mohammed, second co supervisor for their awareness training knowledge and research skills in plant tissue culture.I am also grateful Dr. Prof El-Sadig Hassan El-Sadig and Dr Ashraf Izzeldin Shegidi member of examination committee for their awareness and constructive criticism. Deep special thanks gratitude and approach appreciation is extended to the Date Palm Technology Co LTD. Staff. I am indebted to my colleagues and friends who offered some help in one way or another during conduction of the experiment and not mentioned here in.

List of tables

Table (1) Effect of sucrose concentrations on the length of primary root explants of date palm Mishrig Wad laggai cultivar.....	60
Table (2) Effect of adenine sulfate concentrations on the length of primary root explants of date palm Mishrig Wadlaggai cultivar.....	62
Table (3) Effect of activated charcoal concentrations on the length of primary root explants of date palm Mishrig Wadlaggai cultivar.....	64
Table (4) Effect of agar concentrations on the length of primary root explants date palm Mishrig Wadlaggai cultivar	66
Table (5) Effect of BA concentrations in 1X MS medium on the length of primary root explants of date palm Mishrig Wadlaggai cultivar.....	69
Table(6) Effect of BA concentrations on secondary roots formation on root explants of date palm Mishrig Wadlaggai cultivar.....	71
Table (7) Effect of NAA concentrations in 1X MS medium on the length of primary root explants of date palm Mishrig Wadlaggai cultivar.....	72
Table (8) Effect of NAA concentrations on secondary roots formation on root explants of date palm Mishrig Wadlaggai cultivar.....	74
Table (9) Effect of BA and NAA combination in 1X MS medium on the length of primary root explants of date palm Mishrig Wadlaggai cultivar.	76
Table (10) Effect of BA and NAA combination on secondary roots formation on root explants of date palm Mishrig Wadlaggai cultivar.....	80
Table (11)Effect of BA and NAA concentrations in ½ MS- medium on the length of primary root explants of date palm Mishrig Wadlaggai cultivar.	82
Table (12) Effect of BA and NAA combination in ½ MS-strength media on secondary roots formation on root explants of date palm Mishrig Wadlaggai cultivar.....	84

Table (13) Effect of BA and IAA combination in 1X MS medium on the length of root explants of date palm Mishrig Wadlaggai cultivar.....	85
Table (14) Effect of BA and IAA combination in 1X MS-strength media on secondary roots formation on root explants of date palm Mishrig Wadlaggai cultivar.....	89
Table (15) Me Effect of kinetin in MS medium on the length of primary root explants of date palm Mishrig Wadlaggai cultivar.....	90
Table (16) Effect of kinetin in MS-strength media on secondary roots formation on root explants of date palm Mishrig Wadlaggai cultivar.....	92
Table (17) Effect of kinetin and IAA combination in 1X MS-strength media on primary root explants of date palm Mishrig Wadlaggai cultivar.....	93
Table (18) Effect of kinetin and IAA combination in 1X MS-strength media on secondary roots formation on root explants of date palm Mishrig Wadlaggai cultivar.....	96
Table (19) Effect of kinetin and NAA combination in 1X MS-strength media on secondary roots formation on primary root explants of date palm Mishrig Wadlaggai cultivar.....	97
Table (20) Effect of kinetin and NAA combination in 1X MS-strength media on secondary roots formation on root explants of date palm Mishrig Wadlaggai cultivar.....	98
Table (21) Effect of 2ip and 2. 4. 5-T combination on embryos, color, shoots and roots formation on plantlets regeneration from shoot tips explants callus in date palm Barhee cultivar.....	101
Table (22) Effect of BA and NAA combination in liquid media on embryos, color, shoots and roots formation on plantlets regeneration from shoot tips explants callus of date palm Barhee cultivar.....	103
Table (23) Effect of BA and NAA or 4CPA combination on embryos, color, shoots and roots formation on plantlets regeneration from female immature floral parts explants callus of date palm Barhee cultivar.....	105

Table (24) Effect of BA and NAA or 4CPA combination in liquid media on embryos, color, shoots and roots formation on plantlets regeneration from female immature floral parts explants callus of date palm Barhee cultivar.....109

Table (25) Effect of BA and NAA or 4CPA combination on embryos, color, shoots and roots formation on plantlets regeneration from roots tips explants callus of date Mishrig Wad laggi cultivar.....111

Table (26) Effect of BA and NAA or 4CPA combination in liquid media on embryos, color, shoots and roots formation on plantlets regeneration from roots tips explants callus in date palm Mishrig Wad laggi cultivar.....115

Table (27) Effect of BA and NAA or 4CPA combination on embryos, color, shoots and roots formation on plantlets regeneration from roots tips explants callus of date palm Mishrig Wad laggi cultivar.....117

Table (28) Effect of BA and NAA or 4CPA combination in liquid media on embryos, color, shoots and roots formation on plantlets regeneration from roots tips explants callus of date palm Mishrig Wad laggi cultivar.....119

LIST OF Plato

Plate (1): Callus initiation and induction in MS medium containing 100 mg/l 2, 4, 5-T and 3.0 mg/l 2ip from shoot tips explants Barhee cultivar.....	162
Plate (2): Callus formation growth in MS medium containing 50 mg/l 2, 4, 5-T and 3.0 mg/l 2ip.....	163
Plato (3): Callus maintained in MS medium containing 10 mg/l BA and 10 mg/l 4CPA to formation nodulate callus and somatic embryos after two months on subculture on medium.....	164
Plate (4): Response of callus from shoot tips explants of Barhee cultivar in MS medium containing 3.0 mg/l BA and 0.3 mg/l NAA of plantlets regenerated and somatic embryos formation after 6 weeks on subculture medium.....	165
Plate (5): Shoots regenerated from callus from shoot tips explants of Barhee cultivar in MS medium containing 0.1 mg/l NAA form shoot tips explants of Barhee cultivar.....	167
Plate (6): Developed of shoots regenerated and preceding roots in MS medium containing 0.1 mg/l NAA form shoot tips explants of Barhee cultivar.....	168
Plate (7): Callus initiation and induction in MS medium containing 100 mg/l 2, 4, -5T and 3.0 mg/l 2ip from female floral explants of Barhee cultivar.....	169
Plate (8): Callus maintained in MS medium containing 10 mg/l BA and 10 mg/l 4CPA to formation nodulate callus after two months on subculture on medium.....	170

Plate (9): Callus maintained in MS medium containing 10 mg/l BA and 10 mg/l 4CPA to formation nodulate callus somatic embryos after two months on subculture on medium.....	171
Plate (10): Response of callus from female floral explants of Barhee cultivar in in MS medium containing 3.0 mg/l BA and 0.3 mg/l NAA after 6 weeks on subculture medium.....	172
Plate (11): Cultivar overlaid by a unified liquid MS medium containing 3.0 mg/l BA Response of subculture from female floral explants of Barhee and 0.3 mg/l NAA after 6 weeks on subculture medium	173
Plate (12): Shoots regenerated and somatic embryos growth on basal MS medium from callus of female immature floral explants of Barhee cultivar.....	174
Plate (13): Shoots regenerated and somatic embryos growth on basal MS medium from callus of female immature floral explants of Barhee cultivar.....	175
Plate (14): Shoots regeneration from callus from female immature floral explants of Barhee cultivar on MS basal medium.....	176
Plate (15): Shoots regenerated from callus from female immature floral explants of Barhee cultivar in MS medium containing 0.1 mg/l NAA.....	177
Plate (16): Developed shoots regeneration and preceding roots in MS medium containing 0.1 mg/l NAA from female floral explants of Barhee cultivar.....	178
Plate (17): Callus initiation in MS medium containing 100 mg/l 2, 4, 5-T and 3.0 mg/l 2ip from shoots tips explants of Mishrag Wad Laggai cultivar...	178
Plate (18): Callus formation and growth in MS medium containing 50 mg/l 2, 4, 5-T and 3.0 mg/l 2ip.....	179

Plate (19): Callus maintained in MS medium containing 10 mg/l BA and 10 mg/l 4CPA after two months on subculture medium.....	180
Plate (20): Callus maintained in MS medium containing 10 mg/l BA and 10 mg/l 4CPA after two months on subculture medium and formatted of nodulate callus.....	181
Plate (21): Response of callus from shoot tips explants of Mishrag Wad Laggi cultivar in MS medium containing 3.0 mg/l BA and 0.3 mg/l NAA after 6 weeks on subculture medium	182
Plate (22): Response of subcallus from shoot tips explants of Mishrag Wad Laggai cultivar overlaid by a unified liquid MS medium containing 3.0 mg/l BA and 0.3 mg/l NAA after 6 weeks on subculture medium.....	183
Plate (23): Shoots regenerated from callus from shoots tips explants of Mishrag Wad Laggai cultivar in MS medium containing 0.1 mg/l NAA	184
Plate (24): Developed shoots regenerate and preceding roots in MS medium containing 0.1 mg/l NAA form shoot tips explants of Mishrag Wad Laggai cultivar.....	185
Plate (25): Initiation of roots for callus induction and formation in MS medium form Mashrig Wad Laggai cultivar.....	186
Plate (26): Callus induced in initiation MS medium containing 100 mg/l 2, 4, 5-T and 3.0 mg/l 2ip after 3-4 months on subculture medium.....	187
Plate (27): Callus formation growing in MS medium containing 50 mg/l 2, 4, 5-T and 3.0 mg/l 2ip.....	188
Plate (28): Callus maintained in MS medium containing 10 mg/l BA and 10 mg/l 4CPA after two months on subculture medium.....	189
Plate (29): Response of callus from roots explants of Mishrig Wad Laggai cultivar in MS medium containing 3.0 mg/l BA and 0.3 mg/l NAA after 6 weeks on subculture medium	190

Plate (30): Response of subculture callus from roots explants of Mishrag Wad Laggai cultivar overlaid by a unified liquid MS medium containing 3.0 mg/l BA and 0.3 mg/l NAA after 6 weeks on subculture medium.....191

Plate (31): Shoots regeneration from callus roots explants of Mishrag Wad Laggai cultivar.....191

Plate (32): Developed shoots regeneration and preceding roots form roots explants callus in MS medium containing 0.1 mg/l NAA Mishrag Wad Laggai cultivar.....192

LIST OF Figures

- Figure (1)** Effect of different concentrations of sucrose on primary root explants length (cm) from Mishrig Wad Laggai cultivar.....61
- Figure (2):** Effect of adenine sulfate concentrations (mg/l) on primary root explants length (cm) of Mishrig Wad Laggai cultivar.....63
- Figure (3):** Effect of activated charcoal (g/l) concentrations on primary root explants length (cm) of Mishrig Wad Laggai cultivar.....65
- Figure (4):** Effect of agar (g/l) concentrations on the length (cm) of primary root explants of Mishrig Wad Laggai cultivar.....67
- Figure (5):**Effect of BA concentrations(mg/l) on primary root explants length (cm) of Mishrig Wad Laggai cultivar.....70
- Figure (8):**Effect of NAA concentrations(mg/l) on length (cm) of secondary root formation of Mishrig Wad Laggai cultivar.....75
- Figure (9):**Effect of BA + NAA combinations on the elongation of primary root explants of Mishrig Wad Laggai cultivar.....77
- Figure (10):**Effect of BA + NAA combinations on number of the secondary root explants of Mishrig Wad Laggai cultivar.....81
- Figure (11):**Effect of the combinations of BA + NAA on the length of the primary root explants in $\frac{1}{2}$ MS salts media.....83
- Figure (13):**Effect of BA + IAA combinations on primary root explants length (cm) of Mishrig Wad Laggai cultivar.....86
- Figure (15):**Effect of Kinetin concentration (mg/l) on the elongation primary root explants of Mishrig Wad Laggai cultivar.....91
- Figure (17):**Effect of combinations kinetin + IAA on primary root explants elongation of Mishrig Wad Laggai cultivar.....94

Figure (21):Effect of the 5 regeneration MS media on the callus from shoot tips explants on somatic embryos color and number of regenerated shoots of Bahee cultivar.....102

Figure (22):Effect of the 5 regeneration MS media overlaid by 5ml/culture of MS liquid medium containing 3.0 mg/l BA + 0.3 mg/l NAA on the callus from shoot tips explants on somatic embryos color and number of regenerated shoots of Bahee cultivar.....104

Figure (23):Effect 3 regeneration MS media on somatic embryos color and number of regenerated shoots from callus of female floral explants of Barhee cultivar.....106

Figure (24): Effect of the 3 regeneration MS media overlaid by 5ml/culture of MS liquid medium containing 3.0 mg/l BA + 0.3 mg/l NAA on the callus from shoot tips explants on somatic embryos color and number of regenerated shoots and number of root formation of Bahee cultivar.....110

Figure (25): Effect 3 regeneration MS medium on somatic embryos color and number of regenerated shoots from callus of shoot tips explants of Mishrig Wad Laggai cultivar.....112

Figure (26): Effect of the 3 regeneration MS media overlaid by 5ml/culture of MS liquid medium containing 3.0 mg/l BA + 0.3 mg/l NAA on the callus from shoot tips explants on somatic embryos color and number of regenerated shoots and number of root formation from Mishrig Wad Laggai cultivar.....116

Figure (27): Effect 3 regeneration MS medium on somatic embryos color and number of regenerated shoots from callus of root tips explants of Mishrig Wad Laggai cultivar.....118

Figure (28): Effect of the 3 regeneration MS media overlaid by 5ml/culture of MS liquid medium containing 3.0 mg/l BA + 0.3 mg/l NAA on the callus from root tips explants on somatic embryos color and number of regenerated shoots and number of root formation from Mishrig Wad Laggai cultivar120

Abstracts

Studies were conducted at the Tissue Culture Laboratory, Date Palm Technology Company, Shambat, Khourtom, to determine the morphogenic potential of variety of explant types obtained from field – grown date palm (*Phoenix dactylifera L.*) Trees. In the first experiments three types of root segment namely proximal, median and distal one cm long were excised from field – grown mature trees of Mishrig Wad Laggi cultivar and cultured on Al-Dalaigan (1995) medium supplemented with varies concentrations and combinations of benzyl adenine (BA) or kinetin, naphthalene acetic acid (NAA) , or indole-3-acitic acid (IAA) alone and in combination were tested. The results showed that the low concentrations of both BA or Kin 0.1-1.0 mg/l gave high values for elongation and growth responses measured of primary roots for basal and distal root segment. Also low concentrations of BA and kinetin was best to formed secondary roots. High BA concentrations (>3.0 mg/l) in combination with 0.3 mg/l NAA was equally suitable for the growth and development of primary root of basal root but inhibited the secondary roots formation for basal root segments, similar result were obtained with kinetin combination with NAA or IAA in low concentrations for both basal or distal root segments. High kinetin concentrations at 3.0 mg/l or 1.0 mg/l gave best increase length of primary basal roots and only 3.0 mg/l kinetin induced secondary roots formation on distal root explants.

In the second group of experiments the morphogenic response of shoot tips and root apices and for immature female floral explants excised from mature field grown date palm trees was Mishrig Wad Laggi and Barhee cultivar was evaluated. MS medium supplemented with 100 mg/l

trichlorophenoxy acetic acid (2,4,5-T) and 3.0 mg/l isopentyl adenine (2ip) was used as initiation medium for callus induction for all types explants. Various growth regulators type combination were tested as to their effect on callus induction, maintenance and somatic embryogenesis and regeneration. The physical state of the nutrient media were also assessed. Best callus maintenance was achieved on MS medium contained 10 mg/l of each BA and parachlorophenoxy acetic acid (4CPA) where as embryogenesis was successfully obtained on liquid MS medium supplemented with 3.0 mg/l BA + 0.3 NAA and embryo maturation germination was efficient on also 3.0 mg/l + 0.3 NAA. Plantlets regenerated successfully rooted were transferred to MS medium contained 0.1 mg/l NAA.

اجريت هذه الدراسة بمعمل زراعة الأنسجة بشركة تقانة النخيل المحدودة بشمبات الخرطوم بحرى لدراسة و تحديد اوساط غذائية مثلي لزراعة انسجة نخيل التمر في الانايب لعدة اجزاء نباتية تم الحصول عليها من اشجار نخيل بالغة نامية في الدقل. تم اولاً اجراء تجارب على ثلاثة اجزاء مختلفة شملت الجزء القمي من الجذور : وسط الجذر: و قاعدة الجذر. فصلت من جذور الصنف "مشرقي ود لقاى" حيث تمت زراعتها بعد تعقيمها سطحياً على وسط الدليقان الغذائي (1995) ومن ثم اضافة تراكيز مختلفة من منظمات النمو الأتية: البنزايلى ادانين مفرد او مضافاً اليه نافثلين حمض الخليك (NAA) او اندول حمض الخليك (IAA), الكاينتين مفرد او مضافاً اليه نافثلين NAA او IAA. اظهرت النتائج ان اضافة ال (BA) مع NAA فى تراكيز منخفضة (1- 0.1 ملغ /لتر) اعطت اكبر زيادة فى اطوال قطع الجذور ويكويين الجذور الثانوية لكل من الأجزاء النباتية الثلاثة. شملت التجارب الأخرى انشاء الكاوس وتكوين وانبات الأجنة الجسدية من كل من القمة النامية و قمة الجذور والتي تم الحصول عليها من الصنف مشرق ود لقاى و الأزهار المؤنثة والقمة النامية للصنف برحى من اشجار نامية بالدقل حيث تم تعقيمها سطحياً ومن ثم زراعتها على وسط غذائى يتكون من وسط مورشيحي واسكوج (1962) مضافاً اليه 100 ملغ /لتر ثلاثى كلورفينوكسى حمض الخليك (T-2,4,5) مع 3 ملغ /لتر ايسوبنتيل ادانين (2ip) لأنشاء الكالوس. وتم نقل الكالوس المتكون الى وسط غذائى يتكون من املاح مورشيحي و اسكوج مضافاً اليه 10 ملغ /لتر من كل من BA و (CPA-4) لتكوين الكالوس الحبيبي، تم نقله لعدة اوساط غذائية تحوى تراكيز مختلفة من منظمات النمو لمعرفة تأثيرها على تكون و تكشف الأجنة الجسدية وتطورها الى نباتات و ثم تجديرها لتكون فسائل نخيل. اظهرت النتائج ان اضافة 100 ملغ /لتر من (T-2,4,5) مضافاً اليه 3 ملغ /لتر (2ip) الأفضل فى انشاء و تكوين الكالوس. وتم نقله لوسط غذائى يحتوي 10 ملغ /لتر BA و (CPA-4) لتكون الكالوس الحبيبي من ثم النقل الى وسط غذائى سائل يحتوى على 3 ملغ /لتر BA و 0.3 ملغ /لتر NAA مع املاح مورشيحي واسكوج حيث اعطى اعلي النتائج فى زيادة عدد الأجنة الجسدية المتكونة و تكشفها وتطورها الى نباتات كاملة. تم نقلها الى وسط غذائى يحتوى

على املاح مورسيجي واسكوج مضافا اليه 0.1 ملغ /النر NAA لتجذيرها.

LIST OF CONTENTS

Acknowledgement.....	iii
List of tables.....	iv
List of Plato.....	vii
List of Figures.....	x
Abstracts.....	xii
1. Introduction.....	1
1. 1. Background.....	1
1. 2. Objective.....	4
2. Literature review.....	5
2.2 Botanical description.....	8
2.2.1 Vegetative organs.....	8
2.2.1.1 Root system.....	8
2.2.1.1.1 Root morphology and distribution.....	8
2.2.1.1.1.1. Primary roots.....	8
2.2.1.1.1.2. Tertiary roots.....	9
2.2.1.2. Trunk.....	10
2.2.1.3. Leaves.....	12
2.2.1.4. Inflorescence / Flowers.....	14
2.2.1.5. The date palm Fruits.....	15
2.2.1.5.1. Hababouk stage.....	16
2.2.1.5.2. Kimri stage.....	16

2.2.1.5.3. Khalal stage.....	16
2.2.1.5.4. Rutab stage.....	17
2.2.1.5.5. Tamar stage.....	18
2.3. <i>Climate</i>	18
2.3.1. Temperature.....	18
2.3.2. Humidity.....	19
2.3.3. Wind.....	20
2.4. Soil.....	20
2.5. Geographical distribution of date palm.....	21
2.6. Date palm nutrition value.....	22
2.7. Propagation.....	22
2.7.1. Sexual propagation.....	23
2.7.2. Asexual propagation.....	23
2.7.2.1. Rooting of Off-shoot rapping.....	24
2.7.2.2. Tissue culture.....	26
2.7.2.2.1. Explants type and source.....	28
2.7.2.2.1.1. Sexual zygotie embryos.....	28
2.7.2.2.1.2. Shoot tip culture.....	29
2.7.2.2.1.3. Bud culture.....	30
2.7.2.2.1.4. Leaf culture.....	31
2.7.2.2.1.5. Stem culture.....	31
2.7.2.2.1.6. Inflorescence culture.....	31
2.7.2.2.1.7. Root culture.....	32

2.8. Technical Problems limiting micropropagation.....	34
2.7.1. Microbial concentration.....	34
2.7.2. Browning.....	35
2.7.3. Virtification.....	36
2.7.4. Slow growth rate.....	37
2.7.5. True-to-types.....	38
3. Materials and Methods.....	40
3.1. Explants source and type:.....	40
3.1.1. Root explants:.....	40
3.1.2 Shoot tip and immature floral parts explants:.....	41
3.2. Media Formulation:.....	42
3.2.1. Initiation of root culture:.....	42
3.3. 2. Induction of callus:.....	43
3.3. Experimentation:.....	43
3.3.1. Root culture:.....	43
3.3.1.1. The effect of sucrose:.....	43
3.3.1.2. The effect of adenine sulfate (A/S):.....	43
3.3.1.3. The effect of activated charcoal (A/C):.....	43
3.3.1.4. The effect of agar:.....	43
3.3.1.5. The effect of benzyl adenine (BA):.....	44
3.3.1.6. The effect of NAAA:.....	44
3.3.1.7. The effect of combinations of BA and NAA in full strength MS salts:.....	44

3. 3. 1. 8. The effect of combinations of BA and NAA in half strength MS salts.....	44
3. 3. 1. 9. The effect of BA in combination with IAA:.....	44
3. 3. 1. 10. The effect of Kinetin:.....	45
3. 3. 1. 11. The effect of Kinetin in combination with NAA:.....	45
3. 3. 1. 12. The effect of Kinetin in combination with IAA:.....	45
3. 3. 2. Callus induction.....	45
3. 3. 2. 1. Callus initiation:.....	45
3. 3. 2. 2. Induction of nodulated callus:.....	46
3. 3. 2. 2. 1. Test of concentration of 2,4,5-T:.....	46
3. 3. 2. 2. 2 The effect of 10 mg/l BA in combination with 10 mg/l 4-CPA.....	46
3. 3. 3. Plantlet regeneration.....	46
: 3. 3. 3. 1. The effect of BA at the concentration of 3.0 mg/l in combination with 0.3 mg/l NAA in agar solidified medium.....	46
3. 3. 3. 2. The effect of 3.0 mg/l BA and 0.3 mg/l NAA in a liquid medium.....	46
3. 3. 3. 3. The effect of BA at the concentration of 1.0 mg/l and 1.0 mg/l 4-CPA.....	46
3.3. Statistical Analysis.....	47

4. Results

.1. Roots induction and multiplication.....	59
4. 1. 1. Medium composition.....	59
4. 1. 1. 1. Effect of carbohydrates (sucrose).....	59
4. 1. 1. 2. Effect of adenine sulfate.....	59

4. 1. 1. 3. Effect of activated charcoal.....	59
4. 1. 1. 4. Effect of agar.....	59
4. 1. 1. 5.. Effect of the benzyl adenine (BA) concentrations in MS.....	68
4. 1. 1. 7. Effect of the naphthalene acetic acid (NAA) concentrations in MS.....	68
4. 1. 1. 9. Effect of BA and NAA combination in MS.....	78
4.1. 1. 11. Effect of BA and NAA combination in ½ MS salts strength.....	79
4. 1. 1. 13.Effect of BA and IAA combinations	87
4. 1. 1. 15. Effect of Kinetin concentrations.....	87
4. 1. 1. 17. Effect of Kinetin and IAA combinations.....	88
4. 1. 1. 19.Effect of Kinetin and NAA combinations.....	95
4. 2. Regeneration through Callus induction:.....	99
4.2.21.Response of callus from shoot tips explants of Barhee cultivar.....	99
4. 2. 22 Subculture of callus from shoot tips explants of Barhee cultivar...100	
4. 2.23 Response of callus from female floral explants of Barhee cultivar.....	100
4. 2.24.Subculture of callus from female floral explants of Barhee cultivar.....	117
4. 2.25. Response of callus from shoot tips explants of Mishrig Wad Laggi cultivar.....	117
4. 2. 26. Subculture of callus from shoot tips explants of Mishrig Wad Laggi cultivar.....	133
4. 2. 27. Response of callus from root explants of Mishrig Wad Laggi cultivar.....	134

4. 2. 28. Subculture of callus from root explants of Mishrig Wad Laggi cultivar.....	137
5. Discussion.....	121
6. Conclusion and Recommendations.....	134
7. References.....	138
8. Appendix.....	161