PRODUCTION OF PURE LINES IN BROCCOLI AND CABBAGE USING BREEDING METHODS AND BIOTECHNOLOGY

By

MARWA OMAR ARAFEH

B.Sc. Agric. Sci., Fac. Agric., Al Baath Univ., Syria, 2000 M.Sc. Agric. Sci., (Vegetable Crops), Fac. Agric., Cairo Univ., Egypt, 2006

THESIS

Submitted in Partial Fulfillment of the Requirements for the Degree of

DOCTOR OF PHILOSOPHY

In

Agricultural Sciences (Vegetable Crops)

Department of Vegetable Crops Faculty of Agriculture Cairo University EGYPT

2010

APPROVAL SHEET

PRODUCTION OF PURE LINES IN BROCCOLI AND CABBAGE USING BREEDING METHODS AND BIOTECHNOLOGY

Ph.D. Thesis In Agric. Sci. (Vegetable Crops)

By

MARWA OMAR ARAFEH

B.Sc. Agric. Sci., Fac. Agric., Al Baath Univ., Syria, 2000 M.Sc. Agric. Sci., (Vegetable Crops), Fac. Agric., Cairo Univ., Egypt, 2006

Approval Committee

Professor of Vegetable Crops, Fac. Agric., Ain Shams University	
Dr. AHMED ABDEL-MONEIM HASSANProfessor of Vegetable Crops, Fac. Agric., Cairo University	
Dr. MOHAMED ABD El-MAJEED BADAWI	

Date: 5 / 1 / 2010

SUPERVISION SHEET

PRODUCTION OF PURE LINES IN BROCCOLI AND CABBAGE USING BREEDING METHODS AND BIOTECHNOLOGY

Ph.D. Thesis
In
Agric. Sci. (Vegetable Crops)

 $\mathbf{B}\mathbf{v}$

MARWA OMAR ARAFEH

B.Sc. Agric. Sci., Fac. Agric., Al Baath Univ., Syria, 2000 M.Sc. Agric. Sci., (Vegetable Crops), Fac. Agric., Cairo Univ., Egypt, 2006

SUPERVISION COMMITTEE

Dr. MOHAMED ABD El-MAJEED BADAWI Professor of Vegetable Crops, Fac. Agric., Cairo University

Dr. EL-MAHDY IBRAHIM METWALLY Professor of Vegetable Crops, Fac. Agric., Kafr EL-Shikh University

Dr. SAHAR SAMYH TAHA
Assistant Professor of Vegetable Crops, Fac. Agric., Cairo University

Name of Candidate: Marwa Omar Arafeh Degree: Ph.D.

Title of Thesis: Production of Pure Lines in Broccoli and Cabbage

Using Breeding Methods and Biotechnology

Supervisors: Dr. Mohamed Abd El-Majed Badawi

Dr. El- Mahdy Ibrahim Ali Metwally

Dr. Sahar Sameh Taha Mohamed

Department: Vegetable Crops **Approval:** 5 / 1 / 2010

ABSTRACT

Microspore culture is a very important and useful tool in plant breeding for haploid production and has been developed for many years. Broccoli (Brassica, oleracea var. italica) and cabbage (Brassica, oleracea var. capitata) are important cole vegetable crops; conditions for reliable induction of embryogenesis and plantlets from isolated microspores were studied in eight genotypes of broccoli (Hanin, Conde F₁, Baladi, Dellstare F₁, Marathon F₁, Parthenon F₁, Naxos F₁ and Tiburom) and four genotypes of cabbage (Sabayni, Baladi ,Bronzwic and Nadin F₁). The optimum timing for microspore culture was confirmed to be during the mid to late uninucleate stage. For such purpose, four laboratory and one field trials were conducted at the Agric. Exp. Farm, Fac. Agric., Cairo Univ., Giza, Egypt from 2006/2007 to 2008/2009 growing seasons. Broccoli and cabbage genotypes were responsive significantly to embryogenesis and plantlets regeneration. Embryo and plantlets yield were significantly increased in broccoli genotypes by incubation at 32.5 °C for 24 hours, than that incubated at 32.5 °C for 48 hours or 35.5 °C for 24 or 48 hours, while the best temperature-time treatment in cabbage genotypes embryo and plantlets yield was at 32.5 °C for 48 hours. The use of the NLN-13 medium vielded greater number of embryos than ½NLN-13 and B5 media. Parthenon F₁ and Marathon F₁ from broccoli and Nadin F₁ from cabbage genotypes presented a better response to the NLN-13. Microspore culture density on embryo production was evaluated in selected genotypes, microspore plating density was critical for efficient embryonic induction and development, with an optimal plating density of 4×10^4 microspore/ml. which obtained 237.33 embryos/dish and 23.33 plantlets/dish from Parthenon F₁, and 171.00 embryos/dish and 52.67 plantlets/dish from Marathon F1, and for cabbage genotype obtained 23.667 embryo/dish and 14.67 plantlets/dish from Sabayni and 54.33 embryo /dish and 20.00 plantlets/dish from Nadin F_1 at density 3×10^4 microspore /ml. Activated charcoal (0.2 ml) was added to the liquid NLN-13 medium, embryo yield was significantly higher than those cultures without activated charcoal. Regeneration plantlets developed to double haploid plants as new pure lines, which evaluated for field horticultural characters performance.

Key words: broccoli, cabbage, microspore culture, embryogenesis, double haploid, NLN medium, microspore density, pure line.

CONTENTS

	Page
INTRODUCTION	1
REVIEW OF LITERATURE	5
1. Taxonomy and origin of Brassica crops	5
2. Breeding	11
a. Breeding objectives	11
b . Breeding strategies	12
c. Doubled haploid production	13
1. Advantages	13
2. Methods of haploid production	14
3. Production of Brassica sp. through microspore	
culture	16
a . Genotype	16
b . Donor plant physiology	19
c . Microspore pretreatment	20
d. Developmental stage of microspores	21
e. Culture media	24
f. Culture conditions	28
3. Double haploid plants (new pure lines) evaluation	33
MATERIALS AND METHODS	35
1. Production of double haploid plants (pure lines)	
by microspore culture for B. oleracea. var. italica	
(broccoli) and B. oleracea. var. capitata (cabbage)	36
a. Experimental protocol	36
1. First experiment: effect of genotype- media	
composition	36
2. Second experiment: effect of genotype- density	37
3. Third experiment: effect of genotype	
temperature-time	37
4. Fourth experiment: effect of activated	20
charcoalb. Plant materials	38
	39
1. Broccoli genotypes	39

2. Cabbage genotypes	39
c. Standard microspore culture protocol	40
1. Plant growth conditions	40
2. Microspore culture	40
3. Activated charcoal (AC) suspension preparation	41
4. Media preparation	43
5. Planting date	46
d. Experimental design and data analysis	46
2. Plants adaptation	46
3. Cytological studies	47
4. Primary evaluation for horticultural	
characteristics of new broccoli and cabbage DH lines.	47
a. Experiment protocol	47
b. Experimental design and statistical analysis	48
c. Selfed seed production on DH lines	49
d. Evaluation of the DH lines (R ₁)	49
RESULTS AND DISCUSSION	51
1. Production of double haploid plants (pure lines) by	-
microspore culture for B. oleracea. var. italica	
(broccoli) and <i>B. oleracea</i> . var. <i>capitata</i> (cabbage)	51
a. First experiment: effect of genotype-media	
composition	52
1. Effect of broccoli and cabbage genotypes on embryo	
induction and plantlets regeneration	52
2. Effect of media composition on embryo induction and	
plantlets regeneration.	5 3
3. Effect of genotype-media interaction on embryo	
induction and plantlets regeneration	55
b. Second experiment: effect of genotype-density	61
1. Effect of genotype on embryo induction and plantlet regeneration	62
2. Effect of microspores density on embryo induction	U2
and plantlets regeneration	63
3. Effect of genotype-microspores density interaction on	0.0
embryo induction and plantlets regeneration	65
c. Third experiment: effect of genotype temperature-	

time	
1. Effect of broccoli and cabbage genotypes on embryo induction and plantlets regeneration	
2. Effect of temperature-time treatment on embryo	
induction and Plantlets regeneration	
3. Effect of genotype-temperature-time treatment	
interaction on embryo induction and plantlet	
regeneration	
d. Fourth experiment: effect of activated charcoal:	
1.Effect of broccoli and cabbage genotypes on embryo	
induction and plantlets regeneration	
2.Effect of activated charcoal on embryo induction and	
Plantlets regeneration	
3. Effect of genotype-charcoal interaction on embryo	
induction and plantlets regeneration	
2. Plants adaptation	
3.Cytological studies	
3. Primary evaluation for horticultural characteris	
tics of new broccoli and cabbage DH lines.	
a- Evaluation of the DH lines (R_1)	
1. Plant height and days to maturity:	
2. Head color	
3. Head shape	
4. Head diameter and weight	
5. Yield/plant	
SUMMARY	
REFERENCES	
ADADIC CUMMADV	

LIST OF TABLES

No.	Title	Page
1.	Classification of the major <i>Brassica</i> morpho- types (Williams and Hill 1986)	9
2.	Salts Macro and micro elements and vitamin concentrations of the NLN medium	44
3.	Slats of micro and macro elements in Gamborg B5 medium	45
4.	Effect of broccoli genotype on embryo induction and plantlets regeneration	53
5.	Effect of cabbage genotypes on embryo induction an plantlets regeneration	53
6.	Effect of medium on broccoli embryo induction and plantlets regeneration	55
7.	Effect of medium on cabbage embryo induction and plantlets regeneration	55
8.	Effect of broccoli genotype-medium interaction on embryo induction and plantlets regeneration	57
9.	Effect of cabbage genotype-medium interaction on embryo induction and plantlets regeneration	58

No.	Title	Page
10.	Effect of broccoli genotype on embryo induction and plantlets regeneration	63
11.	Effect of cabbage genotype on embryo induction and plantlets regeneration	63
12.	Effect of broccoli microspore density on embryo induction and plantlet regeneration	64
13.	Effect of cabbage microspore density on embryo induction and plantlets regeneration	65
14.	Effect of broccoli microspores genotype-density interaction on embryo induction and plantlets regeneration.	67
15.	Effect of cabbage microspores genotype-density interaction on embryo induction and plantlets regeneration.	68
16.	Effect of broccoli genotype on embryo induction and plantlets regeneration	71
17.	Effect of cabbage genotype on embryo induction and plantlets regeneration	71
18.	Effect of broccoli incubation temperature on embryo induction and plantlets regeneration	73

No.	Title	Page
19.	Effect of cabbage incubation time on embryo induction and plantlets regeneration	73
20.	Effect of broccoli incubation time on embryo induction and plantlets regeneration	73
21.	Effect of cabbage incubation time on embryo induction and plantlets regeneration	74
22.	Effect of broccoli incubation temperature - time interaction on embryo induction and plantlets regeneration	74
23.	Effect of cabbage incubation temperature - time interaction on embryo induction and plantlets regeneration.	74
24.	Effect of broccoli genotype, temperature and time interaction on embryo induction and plantlets regeneration.	76
25.	Effect of cabbage genotype, temperature and time interaction on embryo induction and plantlets regeneration.	77
26.	Effect of broccoli genotype on embryo induction and plantlets regeneration.	81
27.	Effect of cabbage genotype on embryo induction and plantlets regeneration	81
28.	Effect of activated charcoal on broccoli embryo induction and plantlets regeneration	82
29.	Effect of activated charcoal on cabbage embryo induction and plantlets regeneration	82

No.	Title	Page
30.	Effect of broccoli genotype-charcoal interaction on embryo induction and plantlets regeneration	84
31.	Effect of cabbage genotype-charcoal interaction on embryo induction and plantlets regeneration	85
32.	Primary evaluation for horticultural traits of broccoli doubled haploid (DH) new lines and their donor parental (DP)	93

LIST OF FIGURES

No.	Title	Page
1.	Genetic relationships in different <i>Brassica</i> species (modified from U 1935)	7
2.	Microspore stags, mid to late uni nucleate)	42
3.	Length of flower buds 2-3 mm	43
4.	Early pro-embryos after 20- 30 days of culture	51
5.	Embryos after 10 days of transfer to solid regenerated Medium	51
6.	Embryo, Plantlets (after 4 weeks) formation from microspore cultures on B5 solid medium	87
7.	Transfer plants (new lines) to soil	88
8.	Transferred plants (new lines) to soil	88
9.	Metaphase stage of <i>Brassica oleracea</i> phenotypes cell with 2n=2x=18 chromosomes at diploid level	89

ACKNOWLEDGMENT

First of all, prayerful thanks to our merciful God "ALLAH"

My first, and most earnest, acknowledgment must go to my supervisor Prof. Dr. **Mohamed Abdel-Majeed Badawi**. His patient guidance, constant encouragement, support, and invaluable suggestions made this work successful. Big thanks for his excellent advice in science and life.

Big thanks go to Prof. Dr. **El- Mahdy Ibrahim Metwally,** who had brilliant ideas when I needed them most. My sincere thanks for giving me the chance to work with him.

I wish, also, to express my sincere gratitude to Prof. Dr. Sahar Sameh Taha, who guided this work and helped whenever I was in need. She was always there, as a good scientific advisor and as a good friend. I enormously appreciated not only her scientific support, but also her kindness and excellent advice throughout this work.

I am deeply indebted to my committee members, **Prof. Dr. Mohamed Imam Ragab** and **Prof. Dr. Ahmed Abdel-Moneim Hassan** for their time and efforts in reviewing this thesis.

Many thanks to **Prof. Dr. Christian Mouller** (Göttingen-Germany), and **Prof. Dr. Hassan Rashad**, for opening the door of plant breeding tissue culture laboratory to me, and thanks to Dr. **Fathi Hassan** (Hanover Uni., Germany) for his huge support.

I also owe a huge debt of gratitude to **Ahmed abdel-Hadi**, for helping me in statistical analysis. Thanks for his kindness and support.

My most heartfelt, acknowledgments go to my family, my dad..., my mom. My sisters, and my brothers, for their love kindness.... constant encouragement and unconditional support...

At the end, I would like to thank all those people who made this thesis possible and an enjoyable experience for me.

_	Ш							
	00						00	
✓						00		O_ O
								0_
حيو يـة	التقنية ال	، طرق التربية و	استخداد	ک نب ب	□ ئے، ہالا		لات نقبة م	انتاج سلا
	-, ".	, ". , —, G., — (· <i>y</i> -		-) - -	- - - •	۽ سي سند
Pr		n of Pure Li Breeding M			ccoli	and Cal		
] 5	
						<u>]</u> []] [–
		0 0—0	_			<u> </u>		
	010	3922171🛮 🛈([]
_								
_			m_a	arafeh	@hoti	nail.com		
J			m_a	arafeh	@hoti	nail.com		
_			m_a	arafeh	@hoti	mail.com		
			m_a	arafeh	@hoti	_		
الجامعة	الكلية	القسم	m_a	arafeh	hotı@ _ 	<u> </u>		
	<u>الكلية</u> الزراعة	<u>القسم</u> الخضر	m_a		_ <u>-</u> سىم	ا ل ة 		
الجامعة			m_a		ِ [] سم بيد بدوي	<u>الا</u> عمد عبد المج		١_ الأستاد

_

تستخدم تقنية زراعة حبوب اللقاح لإنتاج نباتات أحادية متضاعفة كوسيلة مهمة و فعالة في مجال تربية النبات، يعتبر البروكولي و الكرنب من محاصيل الخضر الهامة. و قد تمت دراسة العوامل المؤثرة على تكوين أجنة أحادية متضاعفة من زراعة حبوب اللقاح و إمكانية إنتاج نباتات أحادية Hanin, Conde F_1 , Baladi Dellstare F_1 , البروكولى متضاعفة في ثمانية طرز وراثية من البروكولى البروكولى المتضاعفة في ثمانية طرز وراثية من البروكولى المتحاط و أربعة طرز وراثية من الكرنب Marathon F_1 , Parthenon F_1 ,Naxos F_1 , Tiburom كانت أفضل مرحلة لاستخلاص حبوب اللقاح ، Sabayni , Baladi , Bronzwic and Nadin F_1 في مرحلة حبة اللقاح وحيدة النواة استجابت كل من الطرز الوراثية المختلفة للبروكولي و الكرنب بشكل معنوى لتكوين الأجنة و إعادة تشكيل النباتات، كما از داد تكوين الأجنة معنوياً عند التحضين على درجة حرارة ٣٢.٥ م لمدة ٢٤ ساعة بالمقارنة بالتحضين على حرارة ٣٢.٥ م لمدة ٤٨ ساعة أو ٥. ٣٥ م المدة ٢٤ ساعة مع استخدام الفحم النشط في وسط الزراعة. بينما كانت أفضل استجابة للزراعة في الوسط NLN-13 وعند كثافة حبوب لقاح ٤×١٠ نحبة لقاح /مل، كانت أفضل النتائج و هي من البروكولي و من Parthenon F_1 من الطراز الوراثي $Parthenon F_1$ من البروكولي و من الكرنب كانت أفضل النتائج من الطراز الوراثي Nadin F_1 من الطراز الوراثي الكرنب كانت أفضل النتائج من الطراز الوراثي المحاص تم إجراء الدراسة السيتولوجية للسلالات الناتجة و اختيار الأحادية المتضاعفة منها كسلالات جديدة ، كما تم اجراء تقييم للصفات البستانية للسلالات الجديدة في الحقل بغية اختيار السلالات ذات الصفات الأفضل و استخدامها في برامج التربية.

الكلمات الدالة: بروكولي، كرنب، زراعة حبوب اللقاح، أحادية متضاعفة، تكوين الأجنة، وسط الزراعة NLN ،كثافة حبوب اللقاح، سلالة نقية.