Kafrelsheikh University
Faculty of Agriculture
Genetics Department

Genetic Studies on Faba Bean Tolerance to *Orobanche*

BY

Shymaa Farag Ahmed Kalboush
B. Sc. (Genetics), Fac. Agric., Kafrelsheikh University, 2007
M. Sc. (Genetics), Fac. Agric., Kafrelsheikh University, 2012

Thesis

Submitted in Partial Fulfillment of the

Requirements for the Degree of

DOCTOR OF PHILOSOPHY

IN

Agricultural Sciences
(Genetics)

(2017)

CONTENTS

Title	Page
Introduction	**1**
Review of Literature	**4**
1- Effect of *Orobanche* infection on faba bean yield and yield components	**4**
2- Combining ability, heterosis and inbreeding depression	**6**
3- Heritability and genetic components	**13**
4- Effect of mutation treatments on faba bean studied traits for the four genotypes	**17**
5- Biochemical analysis	**19**
6- Molecular analysis	**20**
Material and Methods	**27**
Results and Discussion	**44**
Part (A): Study of the response of four faba bean genotypes and their available crosses to *Orobanche* infection. A.1. Performance of parents, their F_1crosses and their F_2 populations	**44**
A.2. Analysis of variance	**45**
A.3. General combining ability	**49**
A.4. Specific combining ability	**50**
A.5. Heterosis and potence ratio	**52**
A.6. Inbreeding effects	**55**
A.7. Genetic variance components and heritability	**56**
A.8. Correlation coefficient among studied traits	**58**
A.9. Molecular diversity assessment	**60**
A.9.1. RAPD analysis	**60**
A.9.2. SSR analysis	**62**
A.9.3. Molecular distance	**64**
A.9.4. Cluster analysis	**67**
A.9.5. Principal coordinate analysis (PCoA)	**68**
A.9.6. DNA barcoding and genotype-specific markers	**71**
Part (B): Effect of mutagenic treatments (radiation and chemicals) on the induction of *Orobanche* tolerant/genotypes. B.1. Response of studied parental cultivars to mutagenic treatments	**75**
B.1.1. Effect of gamma rays doses and EMS concentrations on the studied traits of faba bean.	**75**
B.1.2. Interaction effects of faba bean genotypes and mutagenic treatments on the studied traits in M_2 populations under *Orobanche* infested soil.	**79**
B.2. Biochemical analysis for faba bean parents under different mutagenic treatments	**88**
B.3. Molecular analysis for the faba bean parents under mutagenic treatments	**99**
B.3.1. RAPD-PCR analysis	**99**
B.3.2. SSR-PCR analysis	**102**
B.3.3. Genomic template stability percentage (GTS %) for faba bean parents under different mutagenic treatments	**106**
Summary	**116**
Conclusion	**122**
References	**124**
Arabic Summary	

List of tables

Table No.	Table title	Page
1	Pedigree and reaction to broomrape (*Orobanche sp*.) of studied faba bean genotypes.	**27**
2	List of RAPD primers and their nucleotide sequences.	**28**
3	List of SSR primers and their nucleotide sequences.	**29**
4	Sources of variations and expected mean squares.	**34**
5	Analysis of variance for combining ability and the expected mean squares of method II, model I for each experiment.	**35**
6	Performances of parents and their half-diallel crosses in F_1 and F_2 populations under *Orobanche* infested soil.	**45**
7	Mean square estimates of combining ability analysis in F_1 crosses for all studied traits.	**48**
8	Mean square estimates of combining ability analysis in F_2 populations for all studied traits.	**48**
9	Estimates of general combining ability (GCA) effects for all parents on all studied traits in F_1 crosses.	**49**
10	Estimates of general combining ability (GCA) effects for all parents on all studied traits in F_2 populations.	**50**
11	Estimates of specific combining ability (SCA) effects for F_1 crosses on all studied traits.	**50**
12	Estimates of specific combining ability (SCA) effects for F_2 populations on all studied traits.	**51**
13	Percentages of heterosis relative to mid-parent for all studied traits in F_1 crosses.	**53**
14	Percentages of heterosis relative to better parent for all studied traits in F_1 crosses.	**53**
15	Percentages of potence ratio for all studied traits in F_1 crosses.	**54**
16	Estimates of inbreeding depression (%) in F_2 populations for all the studied traits.	**55**
17	Genetic variance components and heritability of studied traits for F_1 crosses.	**57**
18	Genetic variance components and heritability of studied traits for F_2 populations.	**57**
19	Correlation coefficients among studied traits of faba bean genotypes (combined data).	**59**

Cont.

20	Numbers and types of the amplified DNA bands as well as the polymorphism percentage generated by the five RAPD primers.	**61**
21	Numbers and types of the amplified DNA bands as well as the polymorphism percentage generated by the five SSR primers.	**63**
22	Nei molecular distance between Pairwise *Vicia faba* parents and F_2 populations based on RAPD data.	**65**
23	Nei molecular distance between Pairwise *Vicia faba* parents and F_2 populations based on SSR data.	**65**
24	Nei molecular distance between Pairwise *Vicia faba* parents and F_2 populations based on combined data.	**66**
25	Faba bean genotypes characterized by positive and negative genotype-specific markers and their molecular sizes (bp) using RAPD and SSR analysis.	**73**
26	Days to flowering, plant height, number of branches per plant and number of pods per plant in M_2 populations as affected by faba bean parents and mutagenic treatments under *Orobanche* infested soils.	**76**
27	Number of seeds per plant, seed yield per plant, *Orobanche* spikes/plant and 100-seed weight in M_2 populations as affected by faba bean parents and mutation treatments.	**78**
28	Number of days to flowering of faba bean as influenced by the interaction between genotypes and mutation treatments in M_2 populations under *Orobanche* infested soil.	**80**
29	Plant height of faba bean as influenced by the interaction between genotypes and mutation treatments in M_2 populations under *Orobanche* infected soil.	**82**
30	Number of branches per faba bean plant as influenced by the interaction between genotypes and mutation treatments in M_2 populations under *Orobanche* infested soil.	**83**
31	Number of pods per faba bean plant as influenced by the interaction between genotypes and mutation treatments in M_2 populations under *Orobanche* infested soil.	**84**
32	Number of seeds per plant of faba bean as influenced by the interaction between genotypes and mutation treatments in M_2 populations under *Orobanche* infested soil.	**85**
33	Seed yield per plant of faba bean as influenced by the interaction between genotypes and mutation treatments in M_2 populations under *Orobanche* infested soil.	**86**
34	Number of *Orobanche* spikes per plant as influenced by the interaction between genotypes and mutation treatments in M_2 populations under *Orobanche* infested soil.	**87**
35	Total soluble protein (TSP) banding patterns for Misr1 cultivar and its M_2 populations.	**89**

Cont.

36	Total soluble protein (TSP) banding patterns for Giza843 cultivar and its M_2 populations.	**93**
37	Total soluble protein (TSP) banding patterns for Sakha2 cultivar and its M_2 populations.	**96**
38	Total soluble protein (TSP) banding patterns for Nubaria1 cultivar and its M_2 populations.	**98**
39	Number and types of the amplified DNA bands as well as the polymorphism percentage generated by the five RAPD primers.	**101**
40	Number and types of the amplified DNA bands as well as the polymorphism percentage generated by the five SSR primers.	**105**
41	Changes in DNA-RAPD profile of Misr1 treated with different doses of gamma rays.	**108**
42	Changes in DNA-RAPD profile of Misr1 treated with different concentrations of ethyl methane sulphonate (EMS).	**108**
43	Changes in DNA-RAPD profile of Giza843 treated with different doses of gamma rays.	**110**
44	Changes in DNA-RAPD profile of Giza843 treated with different concentrations of ethyl methane sulphonate (EMS).	**110**
45	Changes in DNA-RAPD profile of Sakha2 treated with different doses of gamma rays.	**112**
46	Changes in DNA-RAPD profile of Sakha2 treated with different concentrations of ethyl methane sulphonate (EMS).	**112**
47	Changes in DNA-RAPD profile of Nubaria1 treated with different doses of gamma rays.	**115**
48	Changes in DNA-RAPD profile of Nubaria1 treated with different concentrations of ethyl methane sulphonate (EMS).	**115**

List of figures

Figure No.	Figure title	Page
1	RAPD profiles for the tolerant parents (Misr1 and Giza843), Sensitive parents (Sakha2 and Nubaria1), their tolerant F_2 bulk [P_1 x P_2(R)], sensitive F_2 bulks [P_1 x P_2 (S)] and other bulks of F_2 (P_1 x P_3, P_1 x P_4, P_2 x P_3, P_2 x P_4 and P_3 x P_4) using OPH-05 RAPD marker.	**60**
2	SSR fingerprint for the tolerant parents (Misr1 and Giza843), Sensitive parents (Sakha2 and Nubaria1), their tolerant F_2 bulk [P_1 x P_2(R)], sensitive F_2 bulks [P_1 x P_2 (S)] and other bulks of F_2 (P_1 x P_3, P_1 x P_4, P_2 x P_3, P_2 x P_4 and P_3 x P_4) using GAII-8 SSR marker.	**63**
3	Dendrogram from the UPGMA grouping analysis, using Nei & Lei coefficient based on (A) five RAPD markers, (B) five SSR markers and (C) combined data in 11 genotypes of faba bean.	**67**
4	Principal coordinate analysis (PCoA) results of the 11 genotypes of faba bean produced by five RAPD markers.	**69**
5	Principal coordinate analysis (PCoA) results of the 11 genotypes of faba bean produced by five SSR markers.	**70**
6	Principal coordinate analysis (PCoA) results of the 11 genotypes of faba bean as combined data.	**71**
7	DNA barcoding for 11 genotypes of faba bean with total number of fragments by using five RAPD primers.	**72**
8	DNA barcoding for 11 genotypes of faba bean with total number of fragments by using five SSR markers.	**74**
9	Total soluble protein banding patterns for Misr1 genotype	**88**
10	Total soluble protein banding patterns for Giza843 genotype	**92**
11	Total soluble protein banding patterns for Sakha2 genotype	**95**
12	Total soluble protein banding patterns for Nubaria1 genotype	**97**
13	**RAPD profiles for:** Misr1 using OPH-02 primer **(A)**, Giza843 using OPH-01 primer **(B)** and Sakha2 using OPH-04 primer **(C)**.	**100**
14	**SSR fingerprinting for:** Misr1 using GAII-8 primer **(A)**, Giza843 using GAII-8 primer **(B)** and Sakha2 using GAII-8 primer **(C)**.	**104**

Summary

This study was carried out at Sakha Agricultural Research Station, Kafrelsheikh governorate, Agricultural Research Center (ARC), Egypt and Genetics Department, Faculty of Agriculture, Kafrelsheikh University during the three planting seasons; 2013/2014, 2014/2015 and 2015/2016. All the possible cross combinations among parental genotypes (Misr1, Giza843, Sakha2 and Nubaria1) were made according to half-diallel to produce six F_1 crosses.

The four parents were treated with three doses of gamma rays (10, 25 and 50 Gy) and three concentrations (0.2, 0.4 and 0.6%) of ethyl methane sulphonate (EMS). M_1 generations, F_1 crosses and their parents cultivated in the second season at 1st of November under normal conditions. Seeds of four parents and their twenty four M_2 generations were sown in Randomized Complete Block Design (RCBD) with three replications under heavy natural infested soil with *Orobanche crenata* seeds. Also, seeds of four parents, their six F_1 crosses and their F_2 cross combinations progenies were sown in RCBD under heavy natural infested soil with *Orobanche crenata* seeds.

Data was recorded on the following traits; number of days to flowering, plant height, number of branches/plant, number of pods/plant, number of seeds/plant, seed yield per plant, number of *Orobanche* spikes/plant and 100-seed weight. Molecular and biochemical markers were used to detect marker associated with *Orobanche crenata* tolerance and/or susceptibility in some half-diallel faba bean crosses and M_2 populations.

The obtained results could be summarized as follow:

A. Results of half-diallel crossing:

1- Analysis of variance, showed that mean squares of genotypes, parents and crosses were significantly different, crosses also were significant for all traits in both generations. Mean squares of GCA and SCA were significant for all traits in both generations, except mean squares due to SCA for pods per plant (F_1) and 100-seed weight (F_2).

- The ratio of GCA/SCA variance was more than unity for number of days to 50% flowering, branches/plant, pods per plant, seeds per plant and seed yield/plant in both generations. This indicates that the additive genetic effect played a major role in inheritance of these traits, while the GCA/SCA ratio was lower than unity for plant height and *Orobanche* spikes/plant in F_2.

2- Combining ability:

- The parental genotypes Misr1 and Giza843 were good general combiners for yield and its components and days to flowering in both generations.

- The parental genotypes Giza843 and Sakha2 behaved as good general combiners for *Orobanche* spikes/plant in both generations. Where, significant negative GCA effects were found.

- Giza843 x Sakha2 and Sakha2 x Nubaria1 crosses had highly significant negative SCA effects for *Orobanche* spikes/plant in both generations. While, the cross Misr1 x Giza843 showed significant negative SCA effects in F_2 only.

3- Heterosis and potence ratio:

- Mean squares of parents *vs* crosses were significant for all traits, except mean squares of parents *vs* crosses for plant height (F_1), branches per plant in F_1 and F_2, number of *Orobanche* spikes/plant (F_1) and seed/plant (F_2)

- The crosses Misr1 x Nubaria1 and Giza843 x Misr1 showed highly or/and significant better parental heterosis for 100-seed weight. The crosses Misr1 x Giza843, Giza843 x Saka2 and Sakha2 x Nubaria1 had highly significant over better parent in negative direction for *Orobanche* spikes/plant.

4- Heritability and inbreeding effects:

- Heritability values in broad sense ranged from 89.57% for 100-seed-weight to 99.41% for days to flowering and from 58.11% for 100-seed weight to 99.41% for seed yield per plant in (F_1) and (F_2), respectively. Heritability estimates in narrow sense ranged from 14.61% for 100-seed weight to 82.66% for number of branches/plant and from 11.38% for 100-seed weight to 95.07% for seed yield/plant in (F_1) and (F_2), respectively.

- The cross Misr1 x Giza843 showed highly significant inbreeding depression for number of branches per plant. The cross Misr1 x Sakha2 had significant highly or significant inbreeding gain for days to flowering, pods/plant, seeds/plant and seed yield/plant.

B. Effects of mutagenic treatments:

- Number of days to flowering was significantly differed among the studied genotypes of faba bean. Nubaria1 cultivar was the latest one

followed by Sakha2, while Giza843 was the earliest cultivar. Both of Misr1 and Giza843 recorded the highest values of plant height and number of branches per plant under *Orobanche* infested soil followed by Sakha2, while the lowest values for both traits were recorded by Nubaria1 (sensitive cultivar to *Orobanche*).

- All studied traits of faba bean were significantly influenced by mutagenic treatments, except number of branches per plant.

- Except 100-seed weight character of faba bean, all studied traits were significantly affected by the interaction between genotypes and mutagenic treatments.

- For days to flowering, Giza843 untreated plants were the earliest in maturity followed by Misr1 control plants without significant difference with Giza843 treated with 25 Gy of gamma rays. On contrast, Nubaria1 untreated plants recorded the highest number of days to 50% flowering.

*** Biochemical analysis:**

- Misr1 genotype and its M_2 populations scored 24 bands of total soluble protein banding patterns. The polymorphism percentage was 95.83%. In respect to the positive-specific genotype markers, the highest value (2) was recorded by resistant bulk of Misr1 treated with 10 Gy gamma rays to *Orobanche* with MWs of (19.15 and 34.67 KDa) and RF (0.814 and 0.619).

*** Molecular analysis:**

- Five RAPD primers were used to study the genetic diversity among four faba bean cultivars and their seven bulks of F_2. A total of 54 amplified DNA fragments with sizes from 113 bp to 1647 bp were obtained, out of

them 44 (81.48%) were polymorphic. Genetic differences and relationships among the four faba bean parents and their bulks of M_2 based on the RAPD primers showed that, the highest value for average of total amplified and number of polymorphic bands (18.5) was scored by OPH-01 primer. The polymorphism percentage for all genotypes was 100% recorded by using the five primers of RAPD.

- Five SSR primers were also used to study the genetic diversity among four faba bean genotypes and their seven bulks of F_2. A total of 26 loci were obtained, out of them 22 (84.62%) were polymorphic. Genetic diversity among the four faba bean parents and their bulks of M_2 based on five SSR data showed that, the primers JF1-AG3 and GA4 showed the highest average of total amplified and polymorphic bands (10.50 and 10.25), respectively. While, the highest average of polymorphism percentages were found by primers GA4, GAII-30, GAII-59 and JF1-AG3.

- The molecular distance (MD) was detected among 11 faba bean genotypes by using RAPD primers. The highest MD (0.491) was found between Nubaria1 and the sensitive bulks from the cross Misr1 x Giza843, while the lowest MD (0.082) was found between Misr1 and Giza843.

- The dendrogram based on combined data showed that three parental genotypes (Misr1, Giza843 and Sakha2) were separated in one sub-cluster, also Misr1 and Giza843 were located at the same molecular distance as shown in the dendrogram based on SSR and RAPD data.

- Based on RAPD and SSR data, the first group of PCoA contained Misr1, Giza843 and Sakha2 cultivars, it was similar to that obtained by UPGMA clustering.

- DNA barcoding based on RAPD data showed that all genotypes gave a total of 343 DNA fragments with an average of 31.18 fragments per genotype. The total number of unique bands was 14.

- In respect to the positive genotype-specific marker, the genotype Misr1 x Giza843 (S) recorded three positive genotypes-specific markers with sizes of 995 bp, 1018 bp and 1584 bp. The genotype Giza843 x Sakha2 exhibited two positive genotype-specific markers with sizes of 896 bp and 1218 bp.

- All treatments recorded low GTS comparing with control plants, the highest GTS% was observed in susceptible bulk of Misr1 treated with 50 Gy gamma rays and 0.4% EMS as compared to control plants.