

## **GENERATION MEAN ANALYSIS OF EARLINESS AND CHOCOLATE SPOT DISEASE RESISTANCE AND QUALITATIVE VARIATION IN THE STORAGE PROTEINS OF THREE *Vicia faba* L. CROSSES**

**Abo Mostafa, R.A.I.\*; W. M. El-Rodeny \* and Aziza M. Hassanein\*\***

**\* Food Legumes Res. Sec. Field Crops Res. Inst., Agric. Res., Center (ARC).**

**\*\* Seed Technology Research Dep., Field Crops Res. Inst., Agric. Res., Center (ARC), Egypt**

### **ABSTRACT**

Production of grain legumes is limiting by the usual array of pathogenic that affect plants. The present study was conducted during 2005/06, 2006/07 and 2007/08 seasons at Sakha Agricultural Research Station (SARS). A quantitative analysis were done by using six generations mating design between five faba bean parental genotypes namely; Rena Mora, Sakha1, Giza 3, Sakha 2 and T.W to produces three crosses namely; Rena Mora x Sakha1, Giza 3 x Rena Mora and Sakha 2 x T.W. The parental genotypes could be arranged in to three groups. The first group included; Rena Mora, Sakha 1 and Sakha 2 where it is considered as the most resistant group to chocolate spot and high yielding ability. The second group included Giza 3 which is moderate resistant to chocolate spot and the third group include T.W as susceptible genotypes with low yielding potentiality. The data revealed that, the parental genotypes and their crosses had the highest value of variation according to relation to chocolate spot reaction and maturity date. Heterosis over mid and better parent for all traits were highly significant except chocolate spot reaction in the two crosses; (Rena Mora x Sakha1) and (Sakha 2 x T.W.) relative to mid parents. And also, for no. of pods/plant in the cross; (Rena Mora x Sakha1) relative to better parent. Potence ratios were exceeded unity for most traits indicating over dominance. On the other hand, the values of this parameter were less than unity in the cross; (Sakha 2 x T.W.) for maturity date, no. of branches/plant, seed yield/plant(g), 100 seed weight and chocolate spot reaction, indicating partial dominance. The inbreeding depression estimates were highly positively significant for no. of pods/plant, no. of seeds/plant and seed yield/ plant(g) in the two crosses (Rena Mora x Sakha1) and (Giza 3 x Rena Mora). The additive type (a) was significant positive or negative values in all of crosses for all traits except in the cross; (Rena Mora x Sakha 1). For dominance effect (d) it was and higher in magnitude than that of additive type of gene effects. The additive x additive gene effect was highly positive significant in most crosses except in the cross; (Rena Mora x Sakha 1) for no. of branches/plant. However, highly significant positive epistatic gene action (ad) was observed in the first cross; (Rena Mora x Sakha 1) for no. of branches/plant, no. of pods/plant, no. of seeds/plant and chocolate spot reaction. Heritability values in broad-sense were generally higher than the corresponding values in narrow-sense in all crosses for all traits. The additive genes seems to play an important role of the inheritance of maturity date, no. of branches/plant, no. of pods/plant and chocolate spot reaction in the cross; Rena Mora x Sakha1, where the differences between broad and narrow-sense heritability were closest. The indirect selection in the progeny of the crosses; Rena Mora x Sakha 1 and Giza 3 x Rena Mora and direct selection in the progeny of the cross (Sakha 2 x T.W.) would be fruitful due to the high values of narrow-sense heritability and the prediction genetic advance in these cases. The fingerprinted by SDS-PAGE of water-

soluble proteins were performed in two crosses. The results revealed that bands number 2, with MWs 192KDa, was found only in cross Giza 3 x Rena Mora and also exist in the parental genotypes Giza 3 and could be considered as specific bands (positive markers) for these cross. These results support the validity of seed protein electrophoresis as a powerful tool for cultivar identification, clarifying taxonomic and evolutionary problems and studying genetic diversity of *Vicia faba*.

**Keywords:** *Vicia faba*, heritability, genetic components, seed proteins electrophoresis

## INTRODUCTION

Faba bean crop has attracted the attention of most plant breeders to improve its yield because the importance of the crop for both human and animal nutrition. chocolate spot, due to the fungus *Botrytis fabae*, is the most destructive leaf disease of faba bean crop in the world. The losses as a result to foliage diseases infection were estimated to be more than 55% for susceptible cultivar Rebaya 40 which was left for natural infection at Sakha (Mohamed *et al.* 1980). Damages caused by foliar pathogens affecting supply and translocation of photosynthates are prime importance. Although chemical control may provide partial protection, it is costly for small farmers, reduces the crops profitability, and is harmful to the environment. Therefore, selecting resistant varieties is an efficient option to control the disease and an appropriate strategy to promote the development of sustainable agriculture. In this regard, formal plant breeding programs have mainly focused on the production of input responsive and broadly adapted cultivars that show high performance over a wide range of environments. Therefore, cultivars offered by formal plant breeding have to be "distinctive, uniform and stable. Reviewed the contributions of plant breeding facing these problems showing resistance to chocolate spot (*Botrytis fabae*) has been identified in ICARDA lines coming from Ecuador (Bond *et al.*, 1994). The improvement of seed yield and yield stability are the primary objectives of most faba bean breeding program, in Egypt; The challenge in breeding faba bean resides mainly in its reproductive system being partial allogamous. The genetic variability within open-pollinated faba bean varieties and the proportion of heterosis occurring under partial allogamy was studied by Ebmeyer and Stelling (1994). Their results showed that 70% of heterosis for grain yield was achieved for crosses between inbred lines of different varieties and that heterosis could be utilized in open pollination. Characterization of the genetic variation in the available germplasm is important for further improvement of crop yield and to impart resistance to biotic and abiotic stresses (Kour and Singh, 2004). The separation of seed protein components in polyacrylamide gels are mostly informative at the species and infra-specific levels (Nei *et al.*, 1979 and Cooke, 1984). Seed protein data have, therefore been applied to study genetic diversity at interspecific levels (Signor *et al.*, 2005; Mustafa *et al.*, 2006; Sammour *et al.*, 2007) and also for cultivar identification (Sammour, 1988, 1990a, 1992; and Thanh *et al.*, 2006). Electrophoretic techniques offer an exceptional opportunity to study the substructure differences in protein among different genotypes. Nevertheless, SDS-PAGE was used to differentiate between *V. faba* cultivars (Stegemann 1983) and to identify

inbred lines (Gates and Boulter 1979). On the other hand, several researchers have found a greater amount of variation in seed storage protein fractions, legumin and vicilin (Gatehouse *et al.*, 1980). The aim of the present study was to study of genetic diversity and inheritance of earliness, chocolate spot disease resistance, and seed yield and its components of faba bean (*Vicia faba* L.) crosses and their parents through the six generations mean analysis.

## MATERIALS AND METHODS

**Field Experiments:** The present study was carried out during 2005/06, 2006/07 and 2007/08 seasons at Sakha Agricultural Research Station (SARS). In the 2005/06 season five parents of faba bean genotypes namely; Rena Mora, Sakha1, Giza 3, Sakha 2 and T.W were grown and crossed under free insect cage at SARS to produce F<sub>1</sub> seeds. Three crosses were produced namely; Cross I (Rena Mora x Sakha1), cross II (Giza 3 x Rena Mora) and cross III (Sakha 2 x T.W.). The origin, Pedigree, disease reaction and other agronomic characters of these materials are presented in Table (1). In the 2006/07, F<sub>1</sub>'s and their parents for each cross plants were sown under wirecages, F<sub>1</sub> plants were selfed and backcrossed to each parent to obtain the F<sub>2</sub>, Bc<sub>1</sub> and Bc<sub>2</sub>. In 2007/08 the six generations of all crosses were tested under natural infection at early sowing dates in the November 1<sup>st</sup> (Early date). The experiment was arranged in a randomized complete block design with three replications. The plants were grown in ridges of two meters long and 60 cm. wide. Hills were spaced 20 cm. apart with one seed per hill. Plots varied in size; 15 rows for F<sub>2</sub>, three rows for Bc<sub>1</sub> and Bc<sub>2</sub> and three rows for P<sub>1</sub>, P<sub>2</sub> and F<sub>1</sub>. All cultural practices were done as usual with ordinary faba bean culture. Data were taken on plants of the six populations in each cross for the following characters:

**Table 1: The Pedigree , diseases reaction and agronomic characters of five parental faba bean varieties used in the present study.**

No.	Parents	Pedigree	Disease reaction	Earliness of Maturity	Agronomic traits	
					Seed coat color	Seed size
1	Rena Mora (R.M)	Introduction from Spain	R	Medium	Violet (B.H*)	Large
2	Sakha1	Giza 716 x 620/283/85	R	Very Early	Light brown (B.H)	Medium
3	Giza 3	461/845/83 x561 /2076 85	MR	Medium	Light brown (B.H)	Medium
4	Sakha 2	X. 952/1265	H.R	Late	Light brown (W.H**)	Large
5	T.W.	Introduction from Sudan	H.S	Earty	White (W.H)	Small

Reaction for chocolate spot :- H.R = Highly resistant, R= Resistant, M.R = Moderate resistant, S= Susceptible and H.S = Highly susceptible. \*B.H = Black Hilum \*\*W.H = white Hilum

maturity date, no. of branches/plant, no. of pods/plant, no. of seeds/plant, weight of 100 seeds, seed yield/plant and chocolate spot reaction under the natural infection. The resistance to chocolate spot caused by (*Botrytis fabae*) diseases were determined as in Table (2) with the adjustment of grading system from 0 to 9 for the increasing lesion percentage of leaf, flower and stem area covered by lesions, according to the scale of Bernier *et al.* (1984).

**Table 2: Rating scale for chocolate spot (*Botrytis fabae*).**

Rate	Description
1	No disease symptom, or very small specks (Highly resistant)
3	Few small discrete lesions (Resistant)
5	Some coalesced lesions with some defoliation (Moderately resistant)
7	Large coalesced sporulating lesions, 50% defoliation, some dead plants (Susceptible)
9	Extensive lesions on leaves, stems and pods: sever defoliation heavy sporulation: stem girdling: blackening and death of more than 80% of plant ( Highly susceptible)

**Statistical analysis:** All the genetic analysis was done using means and the variance for P<sub>1</sub>, P<sub>2</sub>, F<sub>1</sub>, Bc<sub>1</sub>, Bc<sub>2</sub> and F<sub>2</sub> populations. A.B.C and D scaling test of Mather (1949) were used to test the adequacy of the additive-dominance model and also, to study the non-allelic interaction. The Gamble (1962) procedure's was used to estimate the genetic variance of generation mean and gene action (type of gene effects). The variance of each of the genetic variance components was estimated as linear function of the variance of the mean squares. The variance of a mean square was calculated as a given by (Anderson and Bancroft (1952) and estimates the heritability. Heterosis and percentage of inbreeding depression (I.D %) were measured according (Mather and Jinks, 1971). The nature and type of dominance were determined by means of potence ratio method (P) which can be defined according (Smith, 1952). Expected values of genetic advance (GS) was calculated according to Johanson *et al.* (1955) using the selection differential (K) equal 2.06 for 5% selection intensity. Prediction genetic advance as percent of the F<sub>2</sub> mean (GS %) was calculated as given by Miller *et al.* (1958).

**Biochemical identification:** SDS-Polyacrylamide Gel Electrophoresis (SDS-PAGE) total seed protein was extracted from the five *Vicia faba* genotypes. These protein extractions were analyzed using SDS-PAGE according to the method of Laemmli (1970). A dendrogram was constructed through the complete linkage-joining rule. Nei's similarity index (Nei and Li, 1979) was calculated also among the studied genotypes where:

$$\text{Nei's similarity index} = \frac{\text{The No. of bands in common among all genotypes}}{\text{Total No. of bands}}$$

## RESULTS AND DISCUSSION

The data in Table (3) showed the mean performance, variance, mean variance and coefficient of variation for the six populations of the crosses among different traits. Significantly differences were observed among most

genotypes for measured traits. The parental genotypes could be arranged into three groups. The first group, included the genotypes of Rena Mora, Sakha 1 and Sakha 2, which are considered to be the most resistant group to chocolate spot with high yielding ability where the yield values were: 74.43, 65.11 and 71.02, respectively in addition it is had less rating scale value of chocolate spot reaction; 3.17, 3.33 and 3.00, respectively. The second group included Giza 3 which is moderate resistant chocolate spot (5.57). The third group include; T.W as susceptible genotypes (7) accompanied with low yielding ability (54.38). The data in Fig. 1A, showed the relation between six populations of the three crosses and their reactions to chocolate spot (A) and maturity date (B). The low infected genotypes are generally accompanied by relatively better host reaction, and higher percentage of fruiting plants and higher seed yield. Fig. 1B, showed that the genotypes differed in the values of maturity date and can classified these genotypes into three groups; the first one included the very earliness genotypes T.W. and Sakha 1 with 144.00 and 144.83days from the sowing date to full maturity phase, the second group included the moderate value in earliness 155.43 days for Giza 3 and 155.17 days for Rena Mora, and the third group included lateness genotype, Sakha 2 with 165 days. The difference between the studied crosses with respect to chocolate spot resistance and earliness traits could be observed from the fingers 1A and 1B, respectively. Also, significant genetic variance were detected for all traits in the three crosses and therefore, genetic parameters were detected as reported by Khalil *et al.* (1993b) and Attia *et al.* (2006). The data showed also that  $F_1$ 's were intermediate between their parental genotypes for the time required for maturity, while,  $F_2$ 's later than their  $F_1$ 's in all crosses. While back cross were closer to back crosses parent. The  $F_1$ 's means for the yield and its components traits were higher than those three parents, except no. of branches/plant for the combinations of Sakha2 x T.W. and 100 seed weight for all crosses, this was reflected in the appearance of positive highly significant heterosis for these characters (Table 4).

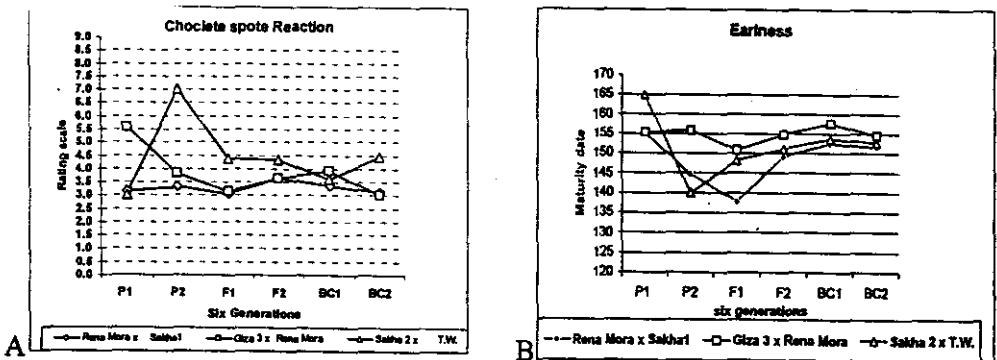


Fig. 1: Frequency distribution curves of chocolate spot reactions for six generations(A) chocolate spot reaction in the left and (B) for earliness in the right.

**Table 3: Mean performance ( $\bar{x}$ ), mean variance ( $S^2_x$ ) and coefficient of variation (C.V.%) for the six populations of the crosses for different traits**

Traits	Cross		P1	P2	F1	F2	BC1	BC2
Maturity date	I	No. plant	30	30	30	180	45	48
		$\bar{X}$	155.17	144.83	137.90	149.18	152.50	151.55
		$S^2_x$	0.083	0.028	0.019	0.192	0.395	0.374
	II	C.V. %	1.04	0.63	0.55	3.80	2.73	2.55
		No.	30	30	30	160	45	48
		$\bar{X}$	155.43	155.90	150.83	154.85	157.68	154.58
	III	$S^2_x$	0.011	0.033	0.028	0.108	0.285	0.259
		C.V. %	0.36	0.66	0.57	2.80	2.19	2.08
		No.	30	30	30	180	45	45
		$\bar{X}$	165.00	140.00	148.27	151.18	153.70	152.73
		$S^2_x$	0.029	0.028	0.016	0.028	0.075	0.074
		C.V. %	0.61	0.88	0.47	1.36	1.20	1.13
Traits	Cross		P1	P2	F1	F2	BC1	BC2
Chocolate spot reaction	I	No.	30	30	30	180	45	45
		$\bar{X}$	3.17	3.33	3.03	3.85	3.35	3.10
		$S^2_x$	0.007	0.010	0.006	0.003	0.009	0.007
	II	C.V. %	14.56	16.40	13.84	18.14	18.57	17.59
		No.	30	30	30	150	45	48
		$\bar{X}$	5.57	3.83	3.17	3.63	3.93	3.00
	III	$S^2_x$	0.008	0.007	0.009	0.005	0.011	0.014
		C.V. %	9.05	12.03	16.76	23.94	17.68	25.04
		No.	30	30	30	180	45	45
		$\bar{X}$	3.00	7.00	4.37	4.33	3.60	4.45
		$S^2_x$	0.011	0.002	0.010	0.005	0.015	0.013
		C.V. %	19.57	7.58	12.73	19.86	22.51	18.05
Traits	Cross		P1	P2	F1	F2	BC1	BC2
No. of branches/plant	I	No.	30	30	30	180	45	46
		$\bar{X}$	5.30	3.27	8.80	5.13	6.58	5.38
		$S^2_x$	0.028	0.014	0.012	0.007	0.016	0.020
	II	C.V. %	16.55	19.58	8.97	19.36	12.84	16.69
		No.	30	30	30	150	45	45
		$\bar{X}$	4.33	6.40	7.33	5.40	3.35	5.10
	III	$S^2_x$	0.021	0.020	0.010	0.065	0.140	0.108
		C.V. %	19.48	7.79	8.54	18.67	19.77	17.65
		No.	30	30	30	160	45	46
		$\bar{X}$	6.20	1.03	5.07	3.43	4.90	5.15
		$S^2_x$	0.012	0.004	0.004	0.005	0.005	0.008
		C.V. %	9.84	17.67	7.21	20.79	10.13	11.25
Traits	Cross		P1	P2	F1	F2	BC1	BC2
No. of pods/plant	I	No.	30	30	30	160	45	45
		$\bar{X}$	15.60	35.20	35.87	22.33	28.80	33.03
		$S^2_x$	0.038	0.019	0.026	0.037	0.070	0.088
	II	C.V. %	6.55	2.16	2.47	10.58	6.22	5.87
		No.	30	30	30	160	45	45
		$\bar{X}$	32.90	15.43	32.30	24.03	28.75	22.50
	III	$S^2_x$	0.021	0.031	0.013	0.047	0.153	0.127
		C.V. %	2.44	5.01	1.68	12.98	8.73	8.22
		No.	30	30	30	160	45	46
		$\bar{X}$	22.93	30.83	33.70	30.35	33.08	34.90
		$S^2_x$	0.041	0.046	0.028	0.031	0.080	0.089
		C.V. %	4.85	3.82	2.72	7.14	4.96	5.42

I (Rena Mora x Sakha1) II (Giza3 x Rena Mora) III (Sakha2 x T.W.)

Table 3:cont.

Traits	Cross		P1	P2	F1	F2	BC1	BC2
No. of seeds/plant	I	No.	30	30	30	160	45	45
		X	63.30	85.50	103.93	76.90	102.23	104.95
		S <sup>2</sup> <sub>x</sub>	0.113	0.096	0.023	0.191	0.422	0.381
		C.V. %	2.91	1.98	0.80	6.95	4.26	3.72
	II	No.	30	30	30	160	45	45
		X	113.90	62.97	116.53	74.30	93.28	72.40
		S <sup>2</sup> <sub>x</sub>	0.022	0.033	0.009	0.117	0.317	0.341
		C.V. %	0.71	1.53	0.54	3.56	2.81	3.11
	III	No.	30	30	30	160	45	45
		X	80.20	70.43	85.00	81.00	84.15	86.20
		S <sup>2</sup> <sub>x</sub>	0.148	0.045	0.009	0.027	0.092	0.077
		C.V. %	2.63	1.65	0.62	2.50	2.42	2.04
Traits	Cross		P1	P2	F1	F2	BC1	BC2
Seed yield / plant(gm)	I	No.	30	30	30	160	45	45
		X	74.43	65.11	114.35	89.48	112.88	108.36
		S <sup>2</sup> <sub>x</sub>	0.076	0.050	0.030	0.133	0.334	0.446
		C.V. %	2.02	1.88	0.83	5.00	3.43	3.90
	II	No.	30	30	30	160	45	45
		X	55.65	74.78	112.66	75.74	87.55	82.10
		S <sup>2</sup> <sub>x</sub>	0.022	0.033	0.009	0.117	0.317	0.341
		C.V. %	1.45	1.33	0.46	5.53	4.31	4.50
	III	No.	30	30	30	160	45	45
		X	71.02	54.38	67.00	64.30	99.96	97.72
		S <sup>2</sup> <sub>x</sub>	0.058246	0.014938	0.021202	0.051629	0.072102	0.13661
		C.V. %	1.86	1.23	1.19	4.33	1.80	2.39
Traits	Cross		P1	P2	F1	F2	BC1	BC2
100-seed weight(gm)	I	No.	30	30	30	160	45	45
		X	117.67	76.19	110.03	113.75	109.74	103.84
		S <sup>2</sup> <sub>x</sub>	0.485	0.166	0.053	0.142	0.420	0.440
		C.V. %	3.24	2.93	1.14	4.05	3.96	4.04
	II	No.	30	30	30	160	45	45
		X	79.16	114.87	100.09	102.98	95.40	112.22
		S <sup>2</sup> <sub>x</sub>	0.044	0.061	0.038	0.114	0.331	0.422
		C.V. %	1.44	1.18	1.06	4.01	4.04	3.66
	III	No.	30	30	30	160	45	45
		X	88.62	64.36	78.96	79.43	118.46	113.57
		S <sup>2</sup> <sub>x</sub>	0.361	0.025	0.032	0.103	0.275	0.285
		C.V. %	3.71	1.35	1.25	4.95	2.97	2.97

I (Rena Mora x Sakha1) II (Giza3 x Rena Mora) III (Sakha2 x T.W.)

**Table 4: Heterosis over mid Parent (M.P) and better parent (B.P), inbreeding depression(I.D.%) and potence ratio for all studies traits in three faba bean crosses.**

Traits	Cross	Heterosis		Inbreeding Depression (I.D%)	Potence ratio (P)
		M.P	B.P		
Maturity date	Rena Mora x Sakha1	-6.89**	-4.86**	-7.63*	-3.22
	Giza 3 x Rena Mora	4.01**	6.63**	3.22	1.64
	Sakha 2 x T.W.	2.19**	9.58**	-1.92	0.33
Chocolate spot reaction	Rena Mora x Sakha1	-6.67	-4.21	-16.89	-2.60
	Giza 3 x Rena Mora	-32.62*	-17.39	-12.64	-1.77
	Sakha 2 x T.W.	-2.96	45.56**	0.96	-0.09
No. of branches/ plant	Rena Mora x Sakha1	58.75**	28.30**	32.68**	2.48
	Giza 3 x Rena Mora	36.65**	14.58	35.80*	1.90
	Sakha 2 x T.W.	40.09**	-18.28**	47.93**	0.56
No. of pods/ plant	Rena Mora x Sakha1	41.60**	2.18	81.10**	1.08
	Giza 3 x Rena Mora	33.66**	-1.82**	34.44**	0.93
	Sakha 2 x T.W.	25.36**	9.30*	11.04	1.73
No. of seeds/ plant	Rena Mora x Sakha1	39.70**	21.56**	35.15**	2.66
	Giza 3 x Rena Mora	31.78**	2.31**	56.84**	1.10
	Sakha 2 x T.W.	12.86**	5.99**	4.94*	1.98
Seed yield/ plant (gm)	Rena Mora x Sakha1	63.90**	53.63**	27.80**	9.57
	Giza 3 x Rena Mora	32.21**	17.78**	48.74**	2.63
	Sakha 2 x T.W.	6.86**	-5.65**	4.20	0.52
100-seed weight (gm)	Rena Mora x Sakha1	13.52**	-6.49**	-3.27	0.63
	Giza 3 x Rena Mora	3.17*	-12.87**	-2.81	0.17
	Sakha 2 x T.W.	3.23	-10.90**	-0.59	0.20



The data presented in Table 4 indicated that heterosis over mid and better parent for all traits were highly in negative or positive direction, except chocolate spot reaction in two crosses (Rena Mora x Sakha1) and (Sakha 2 x T.W.) over mid parents, and also, in no. of pods/plant for cross (Rena Mora x Sakha1) over the better parent. The inbreeding depression estimates were highly significant in positive direction for no. of pods/plant, no. of seeds/plant and seed yield/ plant (g) for the two crosses (Rena Mora x Sakha1) and (Giza 3 x Rena Mora), and also for no. of branches in the crosses (Giza 3 x Rena Mora) and (Sakha 2 x T.W.). However, it was negative significant for maturity date in the cross; (Rena Mora x Sakha1). Potence ratios were exceeding unity for most traits concerning yield and its components, indicating over dominance. On the other hand, the values of this parameter were less than unity but not equal zero in cross; (Sakha 2 x T.W.) for maturity date and chocolate spot reaction, indicating partial dominance. The presence of heterosis over better parent with respect to the cross; (Rena Mora x Sakha1) for maturity date and chocolate spot reaction in negative direction and no. of branches/plant, no. of seeds/plant and seed yield/plant in positive direction, which would indicate that the progeny of this cross could be used in breeding program for maturity, chocolate spot reaction and high yielding ability. Moreover, the progeny of the crosses; (Rena Mora x Sakha1) and (Giza 3 x RenaMora) could be used in breeding program for high yielding potentiality due to the presence of useful heterosis as the results indicated. Inbreeding depression ranged from 27.8% to 61.1% in the cross; (Rena Mora x Sakha1) and from 34.44% to 56.84% in the cross; Giza 3 x Rena Mora for no. of branches/plant, no. of pods/plant, no. of seeds/plant and seed yield/plant. However it might be concluded that one generation of selfing has been sufficient to bring about an inbreeding depression that could negate most of heterosis obtained from crossing for these traits. These results are in harmony with those reported by Abdalla (1977), El-Refaey and Radi (1991), Hendawy *et al* (1994), El- Hosary *et al.* 1997; El-Hady *et al.* (1998), Toker (2004) and Attia *et al.* (2006).

The estimated values of different scaling test according to Mather (1949) and Hayman and Mather (1955), as well as six-parameters describing the nature of gene action and their test of significance according to Gamble (1962), for all studied traits are presented in Table(5). (A) and (B) and tests provides evidence of all types of non-allelic gene interaction. The significance of C scale suggests (dd) type of epistasis. The significant D scale reveals (aa) gene interaction, significance of C and D scales indicates (aa) and (dd) type of gene interaction. The test of adequacy of scales is important because in most cases the estimation of additive and dominance components of the variance are made assuming absence of gene interaction. The values of A, B, C, and D should significantly differ than zero within the Limits of their stander error. However, the results (Table 5) indicated that the values of scaling tests were significantly differ than zero for all studied traits in all crosses, except maturity date of the second cross, indicating that the additive-dominance model is inadequate to interpret the gene effects (Mather, 1949).

Table 5: Mother's scaling test for average addictiveness, Gamble's parameters for determining gene action, broad (H) and narrow heritability( $h^2_n$ ), Genetic advance (Gs %) as percentage of F<sub>2</sub> means and phenotypic variance components for three faba bean crosses for all studied traits.

Traits	Maturity date			Chocolate spot Reaction			No. of branches/plant			No. of pods/plant		
Genotypes	I	II	III	I	II	III	I	II	III	I	II	III
I-Scaling test:												
A	16.03**	-1.92	4.27**	0.33	0.85**	-3.17**	3.08**	-7.03**	3.70**	5.63**	-7.70**	9.52**
B	20.47**	-0.58	21.88**	0.18	-2.73**	1.53**	-1.35**	-1.47**	-0.97**	-5.12**	-2.73**	5.27**
C	25.10**	-7.60**	18.00**	2.21**	-1.23*	-0.43	-1.67*	-3.80**	-3.67**	-33.43*	-16.83**	0.23
D	-5.70**	-2.55	-4.07**	0.85**	0.33	0.60	-1.70**	2.35**	-3.20**	-16.98**	-3.20**	-7.28**
II- Gene Effect:												
M	149.18**	154.85**	151.18**	3.65**	3.63**	4.33**	5.13**	5.40**	3.43**	22.33**	24.03**	30.35**
Additive (a)	0.95	3.10**	0.97*	0.25*	0.93**	-0.85**	1.20**	-1.75**	-0.25*	-4.43**	6.25**	-1.83**
Dominance (d)	1.20	11.27**	11.33**	-1.83**	-2.18**	-1.33	5.92**	-2.73**	7.65**	44.52**	14.53**	21.37**
(aa)	11.40**	5.10**	8.15**	-1.70**	-0.65	-1.20**	3.40	-4.70**	6.40**	33.95**	6.40**	14.55**
(ad)	-2.22*	-0.67	-8.81**	0.42**	0.06	0.65**	0.18**	-0.72**	-2.83**	5.38**	-2.48	2.13**
(dd)	-47.90**	-2.60**	-34.30	1.19	2.53**	2.83**	-5.13**	13.20	-9.13	-34.47**	4.03	-29.33
III- Phenotypic Variance Components:												
Additive variance (D)	50.44	20.19	4.06	0.38	0.92	0.62	0.90	0.74	0.04	8.87	17.66	6.25
Dominance variance (H)	8.97	21.49	5.75	0.07	0.17	0.76	0.06	0.17	0.29	1.23	1.50	1.67
Environmental variance (E)	1.29	0.71	0.72	0.23	0.25	0.24	0.51	0.39	0.21	0.81	0.50	1.14
Ratio of dominance variance	0.42	1.03	1.19	0.44	0.43	1.11	0.26	0.47	2.57	0.37	0.29	0.52
H	95.48	95.58	82.58	48.09	66.88	67.54	47.47	51.01	30.97	85.32	94.75	75.44
Hn	87.69	62.38	48.34	43.86	61.19	41.88	45.86	45.89	7.22	79.79	90.89	66.53
Gs%	9.69	5.17	2.04	0.80	1.09	0.74	0.94	0.85	0.08	3.88	5.84	2.97
I (Rena Mora x Sakha1)      II (Giza3 x Rena Mora)      III (Sakha2 x T.W.)												

Table 5: Cont.

Traits	No. of seeds/plant			Seed yield/plant(gm)			100-seed weight(gm)		
	I	II	III	I	II	III	I	II	III
Genotypes									
I-Scaling test:									
A	37.22**	-43.88**	3.10**	46.30**	-12.34**	78.54**	-8.22**	11.55**	69.34**
B	20.47**	-34.70**	16.97**	27.94**	-44.11**	57.42**	21.47**	9.48**	83.82**
C	-49.07**	-112.73**	3.37**	-10.34**	-92.77**	-2.20	41.10**	17.73**	6.81**
D	-63.38**	-17.08**	-8.35**	-42.29**	-18.16**	-69.08**	13.92**	-1.65	-73.17**
II- Gene Effect									
M	76.90**	74.3**	81.00**	89.48**	75.74**	64.30**	113.75**	102.98**	79.43**
Additive (a)	-2.73**	20.88**	-2.05**	4.52**	5.45**	2.24**	5.89**	-16.82**	4.89**
Dominance (d)	136.28**	62.25**	26.38**	129.16**	63.76**	142.46**	-14.74**	6.38*	148.81**
(aa)	106.75**	34.15**	16.70**	84.58**	36.32**	138.16**	-27.85**	3.31	146.34**
(ad)	8.37**	-4.59**	-6.93**	-0.14	-4.98**	-6.08**	-14.85**	1.03	-7.24**
(dd)	-164.43**	44.43**	-36.77**	-158.82**	20.13**	-274.12**	14.59**	-24.34**	-299.49**
III- Phenotypic Variance Components									
Additive variance(D)	45.97	4.06	1.97	14.26	14.37	13.56	12.02	4.74	14.14
Dominance variance(H)	13.22	17.19	4.36	45.28	38.86	0.08	32.88	53.05	16.69
Environmental variance(E)	2.29	0.65	2.00	1.54	0.64	0.93	6.96	1.41	4.15
Ratio of dominance variance	0.54	2.06	1.49	1.78	1.64	0.08	1.65	3.34	1.09
H	91.90	90.60	50.64	92.24	96.34	87.81	66.92	91.67	72.86
Hn	80.34	29.08	24.06	35.65	40.96	87.54	28.27	13.91	45.81
Gs%	8.85	1.58	1.00	3.28	3.53	5.02	2.69	1.18	3.71

I (Rena Mora x Sakha1) II (Giza3 x Rena Mora) III (Sakha2 x T.W.)

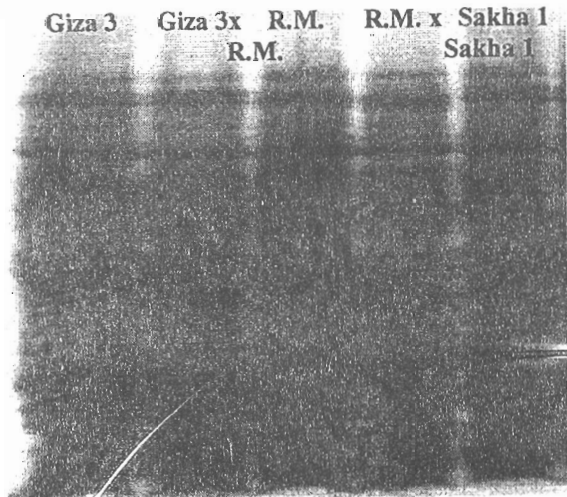
The estimated mean effect parameter ( $m$ ), which reflects the contribution due to the over-all mean plus the locus effects and the interaction of the fixed loci was found to be highly significant for all traits of the three crosses.

The additive type ( $a$ ) was significant in positive or negative direction in all crosses for all traits, except in the cross (Rena Mora x Sakha 1) for maturity date, and also, for dominance effect ( $d$ ) where its highly in magnitude than that of additive type. The additive x additive gene effect was highly positive significant in most crosses; except in the first cross; (Rena Mora x Sakha 1) for no. of branches/plant. However, significant positive epistatic gene action ( $ad$ ) was observed in the first cross; (Rena Mora x Sakha 1) for no. of branches/plant, no. of pods/plant, no. of seeds/plant and chocolate spot reaction. The same trend was found with respect to dominance x dominance ( $dd$ ). While positive or negative significant, were observed for the remaining of the studied characters in these crosses. Generally, at least one of the three types of epistatic gene effects were significant in each crosses for all the studied traits. It could be observed that the significant ( $aa$ ) type of gene effects appeared to be contributed more to the performance of yield and its components than do the significant ( $ad$ ) and ( $dd$ ) gene effects. However, the ( $aa$ ) gene effects were relatively more important than the ( $a$ ) effects, but-generally-less important than the ( $d$ ) effects, moreover, the ( $aa$ ) gene effects are mostly positive, indicating an enhancing effects on performance of the traits ( $s$ ) in question, while the ( $dd$ ) gene effects are negative in most cases giving a diminishing effects which apparently are undesirable form of epistasis. These result were in agreement with those reported by Hejal (1997), El-Refae, 1999; El-Hifny *et al.* (2001), Kalia and Sood (2004), El-Hady *et al.* (2006) and EL-Galaly *et al.* (2008). Heritability values are important to the breeder since it quantifies the expected improvement upon selection. To achieve genetic improvement through selection, heritability must be reasonably high. In the present investigation, the data in Table (5) showed high values of heritability in broad sense ranged from 30.97% to 95.48% for no. branches/plant in cross (Sakha 2 x T.W.) and cross (Rena Mora x Sakha1) for maturity date, respectively. It could be observed that heritability values in the broad-sense were generally higher than the corresponding values in narrow-sense in all crosses for all studied traits, moreover, the crosses differed from one to another in their estimated values of heritability in broad and narrow-sense. However, high heritability values in broad sense were detected in all crosses for maturity date, no. of pods/plant and seed yield/plant. Moderate estimates of broad sense heritability were observed in all crosses for 100 seed weight and chocolate spot reaction, while relatively low estimates were recorded for no. of branches/plant, where the lowest value was detected in the cross;(Sakha2 x T.W.). The additive genes seems to play an important role of the inheritance of maturity date, no. of branches/plant, no. of pods/plant and chocolate spot reaction in the cross; (Rena Mora x Sakha 1) where the differences between broad and narrow-sense heritability were closest. Narrow-sense heritability estimates were relatively high in the cross (Rena Mora x Sakha1) for maturity date, crosses (Rena Mora x Sakha1) and (Giza 3

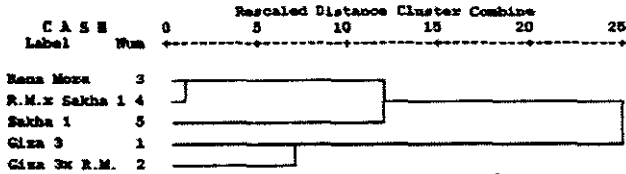
x RenaMora) for no. of pods/plant, cross (Rena Mora x Sakha1) for no. of seeds/plant and cross (Sakha 2 x T.W.) for seed yield/plant, while the lowest of values ( $h_n$ ) were observed in the cross (Sakha 2 x T.W.) for no. of branches/plant, in the crosses (Giza 3 x RenaMora) and (Sakha 2 x T.W.) for no. of seeds/plant and the crosses (Rena Mora x Sakha1) and (Giza 3 x RenaMora) for 100-seed weight. Johanson *et al.* (1955) reported that heritability estimated along with genetic gain are usually more useful in predicting the resultant effect of selection than heritability values alone. From this point of view, the progeny of the cross (Rena Mora x Sakha 1) for maturity date and no. of seeds/plant, the progeny of cross (Giza 3 x RenaMora) for no. of pods/plant and in the progeny of cross (Sakha 2 x T.W.) for seed yield/plant. However, the indirection selection in the progeny of the crosses (Rena Mora x Sakha 1) and (Sakha 2 x T.W.) and direct selection in the progeny of the cross (Sakha 2 x T.W.) would be fruitful due to the high values of narrow-sense heritability and the predicted genetic advance in these cases. These results are in agreement with those reported by El-Hady *et al.* (1997), Kalia and Sood (2004), Attia *et al.* (2006) Al-Ghamdi (2007). and El-Galaly *et al.*(2008).

**Proteins fractionation:**

This study was conducted to study the genetic variations among two crosses and three parental genotypes by using SDS-PAGE electrophoresis for total seed proteins Fig. 2. The data in Table 6 showed that the maximum number of 33 bands and the lowest number of bands (30 bands) was found in the Giza 3 and the rest of genotypes had 31 bands. Bands number 16 and 23 with MWs 83, 58.30 and 63 KDa, respectively, were absent in Giza 3 and Giza 3 x Rena Mora, so it could be considered as common bands the other genotypes. On the other hand, band no. 16 was present in R.M. and absent in Giza3 but the combination between both had not this band. Thus, this finding might due to the switch off genes of this band of protein.



**Figure 2: Electrophoretic patterns of SDS-PAGE for total soluble protein of five *Vicia faba* genotypes.**



**Fig. 3:** Dendrograms based on electrophoretic data of total seed proteins according to Dice using Average Linkage (Between Groups)

**Table(6):** Analysis of protein banding patterns by SDS-PAGE for total soluble protein of five *Vicia faba* genotypes.

Band No.	MW	Genotypes				
		Giza 3	Giza 3 x R.M.	Rena Mora	R.M. x Sakha 1	Sakha 1
1	272	1	1	1	1	1
2	192	1	1	0	0	0
3	187	1	1	1	1	1
4	170	0	1	1	1	0
5	135	1	1	1	1	1
6	117	1	1	1	1	1
7	112	1	1	1	1	1
8	103	1	1	1	1	1
9	98	1	1	1	1	1
10	92	1	1	1	1	1
11	90	1	1	1	1	1
12	87	1	1	1	1	1
13	86	1	1	1	1	1
14	85	1	1	1	1	1
15	84	1	1	1	1	1
16	83	0	0	1	1	1
17	80	1	1	0	0	1
18	78	1	1	1	1	1
19	75	1	1	1	1	1
20	73	1	1	1	1	1
21	69	1	1	1	1	1
22	65	1	1	1	1	1
23	63	0	0	1	1	1
24	59	1	1	1	1	1
25	55	1	1	1	1	1
26	54	1	1	1	1	1
27	51	1	1	1	1	1
28	50	1	1	1	1	1
29	49	1	1	1	1	1
30	48	1	1	1	1	1
31	47	1	1	1	1	1
32	46	1	1	1	1	1
33	45	1	1	1	1	1
<b>Total</b>	<b>33</b>	<b>30</b>	<b>31</b>	<b>31</b>	<b>31</b>	<b>31</b>

Bands number 2, with MWs 192KDa., was found only in cross Giza 3 x Rena Mora and also exist in the parental genotypes Giza 3 and could be considered as specific bands (positive markers) for these cross. And the data in Table 6 reflect the same direction in the seed protein banding patterns especially for band no 2. The data of SDS-PAGE were loaded to the

computer program (SPSS windows version 10) to get a dendrogram for genetic distance and similarity matrix as shown in Table 6 and Figure 3. The dendrogram classified the five genotypes into two groups. The first group included three genotypes were Rena Mora, Rena Mora x Sakha 1 and Sakha 1. While the other group included Giza 3 and Giza 3 x Rena Mora.

Also, in spite of the narrow variation in the number of bands in all genotypes, the Nei's similarity coefficient (Table 7) among all the studied genotypes was small (0.918 to 1.0), indicating a narrow genetic variation between five genotypes for seed proteins. This genetic variation may be indicated in the polymorphism exhibited in the minor bands and in the major bands intensities. These findings are similar with those reported by Sammour (1992), Abd El-Halim, (1994) Zeicl (2003) and Sammour, (2005). Total protein analysis on the importance of heterosis and its usefulness in the improvement of *Vicia faba* (Abd EL-Maksoud et al. 2007). These results support the validity of seed protein electrophoresis as a powerful tool for cultivar identification, clarifying taxonomic and evolutionary problems and studying genetic diversity of *Vicia faba* (Ladizinsky and Hymowitz, 1979; Cooke, 1984; Sammour, 1990a; Signor et al., 2005; Mustafa et al., 2006; Thanh et al., 2006, El-Rodeny, 2006, Abd El-Zaher and Sammour et al., 2007).

**Table 7: Genetic similarity index (Dice measure) among faba bean genotypes based on total seed proteins electrophoretic.**

Genotypes	Giza 3	Giza 3 x R.M.	Rean Mora	R.M. x Sakha 1	Sakha 1
Giza 3					
Giza 3 x R.M.	0.984				
Rean Mora	0.918	0.935			
R.M. x Sakha 1	0.918	0.935	1.000		
Sakha 1	0.951	0.935	0.968	0.968	

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تحليل متوسط الأجيال لصفات التبكير ومقاومة مرض التبقع البني مع التعريف البيوكيمائى للتباين النوعى فى بروتينات البذرة لثلاثة هجن من الفول البلدى رفعت عبدالسلام إسماعيل أبو مصطفى؛ وليد محمد الرضينى عبد الحليم البلقينى\* وعزيزة محمد حسنين\*\*

\* مركز البحوث الزراعية - معهد بحوث المحاصيل الحقلية - قسم بحوث المحاصيل البقولية.  
\*\* مركز البحوث الزراعية - معهد بحوث المحاصيل الحقلية - قسم بحوث تكنولوجيا البذور.  
أجريت هذه الدراسة خلال المواسم الزراعية ٢٠٠٦/٢٠٠٥، ٢٠٠٧/٢٠٠٦، ٢٠٠٧/٢٠٠٦، ٢٠٠٨/٢٠٠٧. فى محطة البحوث الزراعية بمخا/نكر الشيخ وذلك لدراسة السلوك الوراثى لصفات التبكير فى النضج والمحصول وبعض مكوناته والإصابة بمرض التبقع البنى لثلاثة هجن فى الفول البلدى باستخدام نموذج العشائر الستة. وقد استخدم خمسة أباء مقسمة إلى ثلاثة مجموعات طبقا لمدى الإصابة بمرض التبقع البنى هي (أ) أقل حساسية أعلى محصولا (رينامورا سخا ١ سخا ٢)، (ب) متوسط حساسية ومحصول (جيزه ٣) و (ج) أعلى حساسية أقل محصولا (تربل وايت). أجريت التهجينات بين الآباء فى الموسم الأول بالصوبة السلكية للحصول على ثلاثة هجن هي: (سخا ١×رينامورا)، (رينامورا×جيزه ٣)، (تربل وايت×سخا ٢) وبين الآباء والجيل الأول للهجن الثلاثة فى الموسم الثانى للحصول على الهجن الرجعية مع ترك جزء من بذور الجيل الأول للتقبع الذاتى للحصول على بذور الجيل الثانى. فى الموسم الثالث تم زراعة التجربة فى قطاعات كاملة العشوائية فى ثلاثة مكررات شاملة الآباء والجيل الأول والجيل الثانى والهجينون للرجعيين لكل هجين. وأظهرت النتائج الآتى:-

- ١) وجود معنوية عالية بين الآباء والجيل الأول والجيل الثانى والهجن الرجعية لكل الهجن لصفتي التبكير والإصابة بالتبقع البنى وأن الآباء (رينامورا، سخا ١ سخا ٢) كانت أعلى محصول عن الأبوين (جيزه ٣، تربل وايت).
  - ٢) أظهرت قوة الهجين معنوية عالية موجبه وسالبه بناء على متوسط الأبوين و الأب الأفضل لمعظم الصفات فى كل الهجن عدا صفة الإصابة بالتبقع البنى وعدد قرون النبات فى الهجين (سخا ١×رينامورا). وأظهرت النتائج وجود سيادة فائقة لمعظم الصفات فى كل الهجن عدا صفات تاريخ النضج وعدد فروع النبات ومحصول النبات ووزن ١٠٠0 بذره والإصابة بالتبقع البنى كانت سيادة جزئية فى الهجين (سخا ١×رينامورا).
  - ٣) معامل للتربية الداخلية أعطي معنوية عالية لصفات عدد قرون النبات وعدد بذور النبات ومحصول النبات فى الهجين (سخا ١×رينامورا) والهجين (رينامورا×جيزه ٣).
  - ٤) أظهرت النتائج أيضا وجود معنوية أو معنوية عالية موجبه وسالبة لكل من الفعل الجينى المضيف والسيداي والمضيف×المضيف والمضيف×السيداي لمعظم الصفات فى كل الهجن عدا صفات تاريخ النضج وعدد فروع النبات وعدد قرون النبات وعدد بذور النبات والإصابة بالتبقع البنى فى الهجين (سخا ١×رينامورا).
  - ٥) كانت قيمة المكافئ الوراثى بمعناها الواسع اعلى من القيم المناظرة لها للمكافئ الوراثى فى معناه الضيق فى الهجن الثلاثة لجميع الصفات المدروسة.
  - ٦) كان لفعل الجينى المضيف دورا هاما فى توارث صفات التبكير و عدد الفروع وعدد القرون ومرض التبقع البنى فى الهجن (رينامورا X سخا ١) حيث كان الفرق بين قيم المكافئ الوراثى فى معناه الواسع والضيق ضئيلا.
  - ٧) تدل النتائج أن الانتخاب الغير مباشر لبعض مكونات المحصول فى نسل الهجينين (رينامورا X سخا ١) و (جيزه ٣ X رينامورا) وكذلك الانتخاب المباشر لصفة المحصول فى نسل الهجين (سخا ٢ X تربل وايت) سوف يكون فعال ويرجع ذلك للقيم العالية للمكافئ الوراثى فى معناه الضيق وقيم للتقدم الوراثى المتباينة فى هذه الحالات.
  - ٨) تم عمل بصمة وراثية فى إثنين من الهجن حيث عكست النتائج عن وجود حزمة بروتينية رقم ٢ بوزن جزئى ١٩٢ كيلو دالتون وتعتبر حزمة منفردة فى الهجين (جيزه ٣ X رينامورا) ومفرقة عن باقى الهجن وكذلك اعتبارها واسم وراثى إيجابى. من هنا ترجع شرعية استخدام التقريد الكهربى لبروتينات البذرة فى الفول البلدى وسيلة متاحة للتعريف والتقسيم ورصد التطور والتباين الوراثى فى الفول البلدى.
- لذا تشير النتائج إلى وجود أهميه عالية للتباين الوراثى المضيف فى السلوك الوراثى لمعظم الصفات مما يؤكد أهميته فى انتخاب التراكيب الوراثية المرغوبة فى الأجيال الانزلاية المتقدمة وبالتالي سيكون له تأثير فعال فى برامج التربية لتحسين محصول الفول البلدى.