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Inbreeding, outbreeding and RAPD markers studies of faba bean (*Vicia faba* L.) crop

Hazem A. Obiadalla-Ali [a,b], Naheif E.M. Mohamed [c], Abdelsabour G.A. Khaled [d,*]

[a] *Department of Horticulture, Faculty of Agriculture, Sohag University, Sohag 82786, Egypt*
[b] *Plant Production and Protection Department, College of Agriculture and Veterinary Medicine, Qassim University, P.O. 6622, 51452 Buryadh, Saudi Arabia*
[c] *Department of Agronomy, Faculty of Agriculture, Sohag University, Sohag 82786, Egypt*
[d] *Department of Genetics, Faculty of Agriculture, Sohag University, Sohag 82786, Egypt*

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ABSTRACT

Five faba bean genotypes (*Vicia faba* L.) were selfed for two cycles to produce S_1 and S_2 generations. A half-diallel cross was carried out among them in each level of inbreeding (S_0, S_1 and S_2) to obtain 10 F_1 hybrids. Parental materials as well as their respective F_1s were evaluated during the winter season of 2012. All studied traits except total dry seed yield showed significant inbreeding depression after the first generation of selfing (S_1). No further decrease was noticed at the S_2 generation. In the S_1 generation the degree of inbreeding depression was highest for No. of branches/plant (−14.0%) and the least for weight of 100-seeds (−2.7). Some parents showed inbreeding vigor i.e. positive difference between S_2 and S_1 for some traits in S_2 generation. Most studied traits showed significant positive heterosis values over mid-parent. The highest value of heterosis over the mid-parent was detected for total dry seed yield (128.8) and the lowest value of hybrid vigor was shown by weight of 100-seeds (1.2%). Specific combination among the 5 parental genotypes showed the highest value for heterosis for example cross Misr 2 × Giza 429 was the best cross for total dry seed yield, cross Giza 429 × Misr 1 for No. of branches/plant. Giza 429 is the best general combiner for most traits. Some crosses showed heterosis depression i.e. negative heterosis value in some traits. Hybridization among parental genotypes is recommended to be at the S_1 or S_2 generation. Twelve arbitrary primers produced different degrees of genetic polymorphism among the parental genotypes. A total of 65 amplification products were scored polymorphic. The percentage of polymorphic bands detected ranged from 33% to 100% with an average of 66.47%. The average of amplified bands was 5.42 polymorphic bands per primer. A positive, but non-significant, correlation ($r = 0.085$) between Euclidean distance and RAPD distance was observed.

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* Corresponding author. Tel.: +20 1006843428; fax: +20 932280126.
E-mail address: abdelsabour.khaled@agr.sohag.edu.eg (A.G.A. Khaled).

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Introduction

Faba bean (*Vicia faba* L.) is one of the most important legumes crops for human consumption in developing countries and for animal feed, mainly for pigs, horses, poultry and pigeons in industrialized countries. In the Middle East and

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most parts of the Mediterranean, China and Ethiopia, faba bean constitutes one of the main dishes on the breakfast and dinner tables [1]. The most popular dishes of faba bean are Medamis (stewed beans), Falafel (deep fried cotyledon paste with some vegetables and spices), Bissara (cotyledon paste poured onto plates) and Nabet soup (boiled germinated beans) [2]. It is sometimes grown for green manure, but more generally for stock feed. In Egypt and Sudan straw from faba bean harvest fetches a premium and is considered as a cash crop [1]. Wide variation in protein content (20–41%) of faba bean has been reported [3]. Besides being an excellent source of high quality protein, it is considered as a good source of carbohydrate, vitamins and minerals [4].

Improving seed yield and production of faba bean is a priority to meet increased demand from population growth. Production of F_1 hybrid varieties is considered one improvement to achieves these goals [5,6]. Faba bean is a partially allogamous species with about 10–80% natural out-crossing, depending on genotypes and environmental effects [7,8]. The consequences of self-fertilization are important factors to take into account when determining the management of germplasm in species with varied levels of heterogeneity and heterozygosity [9].

Selfing results in reduction in the following: plant height and 100-seed weight [10], number of seeds/pod [11] and yield [12]. Therefore, for a curator, plant breeder and gene bank manager, in addition to the loss of diversity due to random genetic drift, the effect of self-fertilization is one of the issues that must be considered when multiplying and regenerating seeds. The nuclear genome of *V. faba* is enormous, with more than 13,000 Mbp in comparison with the model legume species *M. truncatula*, which is estimated to be 470 Mbp [13]. This large size may be largely explained by a high number of retrotransposon copies [14]. These retrotransposons, microsatellites and genes are the basis of the sequence variability that can be explored in genomes.

Isozymes, RAPDs and RFLPs were used to develop the first meaningful genetic linkage maps for faba bean in F_2 populations [15]. The genetic DNA markers have opened a new vista to study genetic diversity, and these markers have the potential to reveal a large amount of variation with good coverage of the entire genome. Several investigators [16–18], successfully used RAPD molecular markers to study the genetic variability and relationships among accessions, lines and cultivars of faba bean.

The main objectives of this work were to (1) evaluate the effect of hybridization among five faba bean parental genotypes and in particular examine the level of hybrid-vigor for vegetative and reproductive traits in this crop, (2) investigate the effects of changes related to selfing on performance, breeding and germplasm management of our faba bean, and (3) evaluate the genetic diversity among these parental genotypes using random amplified polymorphic DNA (RAPD) marker.

Material and methods

Five local genotypes of faba bean (*Vicia faba*), Misr 2 (P1), Giza 429 (P2), Misr 1 (P3), Giza 40 (P4) and Giza 843 (P5), obtained from Agricultural Research Center of Egypt, were used in the present study. This study was conducted at the Research Farm and biotechnology laboratory of Faculty of Agriculture, Sohag University, Egypt. The soil is reclaimed with top layer (25 cm) of clay-loam. Seeds of the original population (S_0) of the parental genotypes were planted on October 15, 2009. At the flowering stage using hand emasculation and pollination, hybridization was carried out to obtain the 10 possible hybrid combinations (excluding reciprocals). At the same time, five plants of each genotype were isolated and selfed to produce the (S_1) seeds. In the winter season of 2010, the S_1 seeds of each genotype were planted and at the flowering stage a half-diallel cross was undertaken to produce the 10 F_1 hybrid combinations. At the same time some of S_1 plants were selfed to produce the S_2 seeds. In the winter season of 2011, the S_2 seeds of each genotype were planted and at the flowering stage a half-diallel cross were carried out to produce the 10 F_1 hybrid seeds. In the winter season of 2012 seeds of all entries were planted into two experiments. In the first experiment the original population (S_0) and their selfed generations (S_1 and S_2) for all the 5 parental genotypes were randomized in a complete block design with three replicates. In the second experiment the 10 F_1 hybrids produced from the half-diallel cross and their 5 parents for each the 3 levels of selfing (S_0, S_1 and S_2) were randomized in a complete block design with 3 replicates. In both experiments seeds were planted on the southern side of the rows. Each plot consisted of 3 rows 4 m long and 60 cm apart. After complete emergence, plants were thinned to 2 plants per hill spaced at 20 cm. All agricultural practices were as recommended for local commercial production.

The collected data were measured as follows: Number of days to 50% flowering (number of days from planting to flowering date for 50% of plants) and Earliness (number of days from date of planting to maturity for 50% of plants) were recorded during the growth period in each plot; Data on plant height, number of branches per plant, pod setting (number of set pods/number of anthesized flowers) were taken before harvesting as average of 10 plants per plot.

Samples of ten guarded plants were randomly taken from each plot for the following characters: (1) Plant height (cm), (2) Number of branches per plant, (3) Pod setting percentage (number of set pods/number of anthesized flowers). Plants were harvested at full maturity and transferred to the laboratory. Samples of ten plants were also randomly assigned from each plot to determine the following traits: (1) number of pods per plant, (2) weight of 100-seed (g), (3) shellout percentage (weight of dry seeds per plant/weight of dry pods per plant), (4) pod filling Percentage (number of seeds per pod/pod length (cm)), (5) protein content percentage (micro-kjeldahl method used to estimate the total nitrogen. Crude protein was obtained by multiplying the nitrogen percentage by 6.25) and (6) total dry seed yield (kg/ha).

Inbreeding depression was calculated as the percentage decrease in S_1 and S_2 value compared to S_0 and S_1 value as follows:

$$\text{Inbreeding depression } (\%) = \frac{S_1 - S_0}{S_0} \times 100 \text{ and, } \frac{S_2 - S_1}{S_1} \times 100$$

Heterosis expressed by the hybrid in each of S_0, S_1 and S_2 populations was calculated as the percentage increase of the F1 hybrid over its mid-parent values at all levels as follows:

$$\text{Mid-parent heterosis } (\%) = \frac{F_1 - \text{M.P.}}{\text{M.P.}} \times 100$$

$$\text{where, M.P.} = \frac{P_i - P_j}{2}$$

All recorded data were statistically analyzed; analysis of variance for randomized complete block design was carried out according to Gomez and Gomez [19]. Least significance differences (LSD) test was used to detect significant changes of means following each generation of selfing at 0.05 and 0.01 probability levels. Significance of deviations due to mid-parent heterosis was also tested using LSD test at 0.05 and 0.01 probability level.

RAPD markers procedures

Fresh young leaves were harvested and immediately ground in extraction buffer using cetyltrimethylammonium bromide (CTAB) protocol as described by Poresbski et al. [20] with adding 1% polyvinylpyrrolidone (PVP). A total of twenty-four varied 10-mer random primers (Metabion International AG, Germany) were scanned across the five parental genotypes. Amplification was carried out in a DNA Thermal Cycler (Primus 25, Germany) according to the methods described by Williams et al. [21]. The RAPD assay was performed in a 15 µl volume containing 7 µl of Go Taq® Green Master Mix (Promega, Madison, USA), 2 µl of primer 5 pmol, 4 µl of nuclease-free water and 2 µl of 200 ng genomic DNA templates. PCR amplification was programmed for conditions with an initial denaturation cycle at 94 °C for five minutes. The following 40 cycles were composed of the following: denaturation step at 94 °C for 1 min, annealing step at 34 °C for 1 min 30 s and elongation step at 72 °C for 2 min. The final cycle of polymerization was performed at 72 °C for 8 min. The amplification products were electrophoresed in a 1.0% agarose gel stained with 0.2 µl ethidium bromide. The amplified fragments were visualized and photographed using UVP Bio Doc-It imaging system (USA).

Data analysis

The DNA banding patterns generated from RAPD analysis were analyzed by a computer program, Gene Profiler (version 4.03). A Microsoft Excel file was prepared for scoring the data as '1' for matched and '0' for unmatched DNA bands of every genotype. Genetic similarities among genotypes were computed based on the method of Nei and Li [22]. The average of similarity matrix was used to generate a tree for cluster analysis by UPGMA (Unweighted Pair Group Method with Arithmetic Average) method using MVSP (version 3.1) program.

In order to detect patterns of genetic relationship among the parental genotypes, dissimilarity analysis of means of all studied traits was constructed based on the Euclidean distances using the method proposed by Laghetti et al. [23]. The similarity matrix of RAPD was converted to a dissimilarity matrix. A cophenetic matrix was derived from each matrix to test goodness of fit of the clusters by comparing the two matrices using the Mantel test [24]. Finally, the correlation between each distance pair was calculated using computer program NTSYS-pc version 2.1 [25].

Results and discussion

Inbreeding depression

Inbreeding depression (%) after one and two cycles of selfing was estimated for vegetative and reproductive traits (Table 1) and yield and quality traits (Table 2). It is clear that most of the studied genotypes showed significant inbreeding depression in all traits after one cycle of selfing (S_1). These results are in agreement with those obtained by Gasim and Link [10].

Inbreeding depression was extended to the S_2 generation in only one parent for plant height (P2), No. of days to 50% flowering (P5), No. of pod/plant (P3) and shellout percentage (P4), two parents (P1 and P2) in pod filling percentage and three parents (P1, P2 and P4) in protein content percentage (Table 1 and 2). No further significant decrease due to selfing was observed at the S_2 generation in No. of branches/plant (Table 1). Significant positive differences between S_2 and S_1 generations were observed for a number of traits in one or more genotypes, including the following: one genotype (P3) in shellout (%), two genotypes (P1 and P3) in earliness, three genotypes (P2, P3 and P4) in No. of days to 50% flowering and weight of 100-seeds (g) and (P3, P4 and P5) in pod filling, four genotypes (P1, P3, P4 and P5) in plant height and (P1, P2, P4 and P5) in No. of pod/plant and all five genotypes in No. of branches/plant (Table 1 and 2). These results are consistent with those obtained by Hebblethwaite et al. [11]. No significant inbreeding depression in total dry seed yield was detected due to selfing at the S_1 and S_2 generation. This is in contrast to Nassib and Khalil [26] who found significant inbreeding depression in seed yield indicating that observed heterosis in F_1 is a real effect. On the other hand, all genotypes showed

Table 1 Inbreeding depression (%) in some characters of 5 genotypes of faba bean in 3 Levels of inbreeding (S_0, S_1 and S_2).

Genotypes		Inbreeding depression									
		Plant Height		No. of branches/plant		No. of days to 50% flowering		Earliness (no. of days to 50% maturity)		Pod setting percentage	
		S_1 to S_0	S_2 to S_1	S_1 to S_0	S_2 to S_1	S_1 to S_0	S_2 to S_1	S_1 to S_0	S_2 to S_1	S_1 to S_0	S_2 to S_1
P_1		-2.5^{c}	5.8^{d}	-11.4^{b}	5.1^{e}	-3.6^{c}	0.0^{b}	-2.50^{b}	1.10^{b}	-0.08^{e}	0.29^{a}
P_2		-5.9^{b}	-1.6^{a}	-6.7^{c}	4.9^{d}	-8.0^{a}	2.9^{c}	-3.19^{ab}	-0.37^{a}	-5.67^{b}	8.21^{b}
P_3		-11.2^{a}	6.6^{e}	-14.0^{a}	2.3^{b}	-6.0^{b}	5.5^{d}	-3.60^{a}	5.60^{c}	-4.56^{c}	0.38^{a}
P_4		-5.3^{b}	2.1^{c}	-4.3^{d}	3.8^{c}	-1.9^{d}	2.9^{c}	0.00^{c}	0.00^{a}	-10.20^{a}	12.75^{c}
P_5		-3.4^{c}	1.3^{b}	-2.5^{e}	1.3^{a}	-3.2^{c}	-2.5^{a}	-0.68^{c}	-0.34^{a}	4.13^{d}	1.48^{a}
LSD	0.05	0.99	0.743	0.09	0.08	0.65	0.41	0.84	0.92	0.72	1.24
	0.01	1.34	1.84	0.12	0.12	0.88	0.81	1.14	1.12	0.98	1.38
P-value		0.0058	0.0073	0.0014	0.0060	0.0036	0.0053	0.0007	0.0020	0.0021	0.0009

The means with the same letter indicate non significant differences, while the means with different letters indicate significant differences.

Table 2 Inbreeding depression (%) in some characters of 5 genotypes of faba bean in 3 levels of inbreeding (S_0, S_1 and S_2).

Genotypes		Inbreeding depression											
		Total dry seed yield		No. of pods/plant		Weight of 100-seeds		Shellout percentage		Pod filling		Protein Content	
		S_1 to S_0	S_2 to S_1	S_1 to S_0	S_2 to S_1	S_1 to S_0	S_2 to S_1	S_1 to S_0	S_2 to S_1	S_1 to S_0	S_2 to S_1	S_1 to S_0	S_2 to S_1
P_1		3.11[a]	0.79[a]	−4.64[a]	4.97[d]	1.52[e]	0.08[a]	2.32[c]	0.18[b]	−1.86[c]	−2.84[a]	−0.66[d]	−9.29[a]
P_2		−3.08[a]	7.49[a]	−3.89[b]	4.28[c]	−1.04[b]	2.91[c]	−5.78[a]	0.50[b]	−5.33[b]	−0.47[b]	−3.73[b]	−10.19[a]
P_3		−3.55[a]	6.72[a]	−3.20[d]	−0.43[a]	−2.69[a]	4.24[d]	1.98[c]	2.01[c]	4.53[e]	2.36[c]	−8.90[a]	−0.51[c]
P_4		3.45[a]	5.68[a]	0.11[e]	0.34[b]	1.00[d]	0.50[b]	−6.37[a]	−3.82[a]	1.17[d]	3.08[d]	−1.86[c]	−2.67[b]
P_5		−0.18[a]	2.55[a]	−3.54[c]	7.22[e]	−0.09[c]	0.04[a]	−1.29[c]	0.44[b]	−7.54[a]	11.59[e]	−1.03[cd]	−0.24[c]
LSD	0.05	29.74	27.32	0.24	0.26	0.44	0.35	0.53	0.49	0.01	0.01	0.89	0.94
	0.01	40.30	32.12	0.32	0.37	0.59	0.53	0.72	0.76	0.01	0.12	1.21	1.25
P-value		0.140	0.223	0.0002	0.0021	0.0039	0.0069	0.0056	0.0030	0.0009	0.0040	0.0000	0.0003

The means with the same letter indicate non significant differences, while the means with different letters indicate significant difference.

non-significant positive differences between S_2 and S_1 generation for seed yield (Table 2). EI-Hady et al. [27] observed highly significant positive inbreeding depression in the cross Giza 3 × 899/503/89 for 100-seed weight and seed yield. On the other hand significant negative estimates were found in the cross Shambat 104 × Giza 3 for flowering date and in the cross Giza 3 × 899/503/89 for number of seeds per plant. EL-Harty et al. [28] pointed out that some crosses expressed significantly positive inbreeding depression and recorded a range of 10.5–31.4; 8.8–49.9; 10.7–31.2% and 6.8–43.5% for seed yield, pods, seeds per plant and pods per main stem, respectively. Abdalla and Fischbeck [29] reported several inbreeding effects in F_2 population of the hybrids *minor* × *minor*, *minor* × *equina*, *minor* × *major*, *equina* × *major* and *paucijuga* × *eu-faba* types. Inbreeding depression varied in different hybrids and characters. Generally *equina* × *major* hybrids expressed lowest inbreeding depression and high inbreeding depression in F_2 was associated mostly with high heterosis in F_1. Inbreeding gain (high values of F_2 compared to F_1) occurred in certain characters. The latter mostly originated from combinations that showed minus values for heterosis. In contrast, Abdalla and Metwally [30] found that the inbreeding depression in F_2 was not always associated with heterosis in F_1. Gain and not depression may occur in F_2.

Inbreeding depression (ID %) was expressed for all studied characters after the first cycle of selfing (S_1). In this generation there was a wide range of inbreeding depression among characters. The highest inbreeding depression occurred for No. of branches/plant (−14.0%) followed by plant height (−11.2%) and the least for weight of 100-seeds (−2.7%). No further significant decrease due to selfing was observed at the S_2 generation. This could be attributed to that the parental genotypes reach its genetic stability after only one cycle of selfing. Attia [31] observed overall superiority of F_1 hybrids for plant height, pods per plant, seed yield per plant and harvest index that were significantly depressed in F_1's as a result of inbreeding. However, significant inbreeding depression was observed in F_2 for number of branches and seed index. These results were agreement with those obtained by EL-Harty et al. [28] and, Bargale and Billore [32].

Moreover our data showed that some genotypes had significant positive differences between S_2 and S_1. These positive differences could be attributed to the variance of parental interaction with selfing generations. Although inbreeding in faba bean is usually accompanied by reduction in yield [33], some high-yielding inbred lines have been reported by Poulsen and Knudsen [12].

Heterosis

Mid-parent heterosis values (%) were estimated for vegetative and reproductive traits for all the 10 F_1 hybrids in the three levels of inbreeding S_0, S_1 and S_2 (Table 3). Out of 10 crosses only one cross (P1 × P3) at all levels, one cross (P3 × P5) at S_1 level and three crosses (P2 × P4, P3 × P4 and P4 × P5) at S_1 and S_2 levels showed significant positive increase in plant height. Significant mid-parent heterosis for decreased number of days to 50% flowering was detected in five, three and four crosses in S_0, S_1 and S_2 generations respectively, while six hybrids in S_0 and four hybrids in S_1 and S_2 generations exhibited this heterosis in number of days to 50% maturity. It is clear that most crosses showed positive significant mid-parent heterosis for number of branches per plant, pod setting percentage in all levels of inbreeding, except the crosses P2 × P5 and P4 × P5 which exhibited negative significant heterosis in the level S_0 and S_2 generations.

Table 4 presents mid-parent heterosis values (%) for yield and quality traits for the 10 F_1 hybrids in the three levels of inbreeding. Number of pods per plant and seed yield showed positive significant mid-parent heterosis in all crosses for the three level of inbreeding, except the cross P2 × P3 in S_0 and P3 × P4 in S0 and S_1 where heterosis for seed yield was non-significant. The highest values of mid-parent heterosis were detected in the cross P1 × P2 at all levels of selfing for seed yield and in the cross P3 × P4 in the level S_1 and S_2 for No. of pod per plant. These results are in agreement with those obtained by Farag and Afiah [34], who reported significant positive heterosis for a number of traits. With respect to seed yield per plant, seven crosses had significant positive heterotic effects relative to mid and better parents under the two irrigation treatments. Abdelmula et al. [35] studied heterosis and inheritance of faba been under well-watered and dry conditions and found significant mid parent heterosis for yield under dry condition (Yd) and well-watered (Yw) but not for drought tolerance (Yd/Yw). Furthermore the relative heterosis for Yd (52.0%) was greater than for Yw (39.3%).

Significant negative heterosis was noticed in all crosses at all levels of inbreeding for 100 seed weight, except P3 × P4 at S_1 levels. Significant mid-parent heterosis for greater shellout percentage was detected in six hybrid combinations in S_0, S_1

Table 3 Heterosis (%) value over Mid-parents in some characters of the 10 F_1 hybrids of Faba bean in 3 levels of inbreeding (S_0, S_1, and S_2).

Crosses		Heterosis (%) over Mid-parents														
		Plant Height			No. of branches/plant			No. of days to 50% flowering			Earliness (No. of days to 50% maturity)			Pod setting percentage		
		S_0	S_1	S_2	S_0	S_1	S_2	S_0	S_1	S_2	S_0	S_1	S_2	S_0	S_1	S_2
$P_1 \times P_2$		-6.4^{b}	-4.2^{a}	-3.3^{a}	15.2^{j}	23.3^{h}	21.4^{g}	0.4^{e}	3.8^{e}	7.0^{e}	-1.8^{bc}	0.0^{c}	1.5^{e}	13.8^{e}	13.1^{c}	21.9^{e}
$P_1 \times P_3$		5.5^{g}	5.2^{e}	3.1^{c}	11.1^{g}	21.9^{g}	14.0^{e}	6.2^{g}	0.5^{cd}	2.3^{c}	1.1^{e}	0.9^{cd}	-1.2^{c}	5.0^{b}	-0.7^{a}	15.8^{d}
$P_1 \times P_4$		-4.7^{c}	-1.8^{c}	-3.4^{a}	9.0^{f}	15.0^{e}	8.5^{c}	1.9^{f}	1.0^{d}	1.4^{c}	-0.4^{cd}	0.9^{cd}	2.9^{f}	7.3^{d}	-1.2^{a}	10.2^{b}
$P_1 \times P_5$		-3.1^{cd}	-2.8^{abc}	-0.5^{b}	1.8^{c}	16.4^{f}	2.9^{b}	-5.9^{a}	-7.0^{a}	-6.7^{a}	-5.2^{a}	-4.1^{a}	-5.5^{a}	21.9^{g}	25.0^{e}	32.3^{g}
$P_2 \times P_3$		-8.6^{a}	-2.3^{bc}	-3.1^{a}	7.6^{e}	24.7^{i}	22.8^{h}	-6.5^{a}	-0.5^{c}	-5.4^{b}	-2.9^{b}	0.9^{cd}	-2.0^{c}	19.0^{f}	34.0^{h}	27.6^{f}
$P_2 \times P_4$		1.3^{f}	8.3^{f}	8.8^{e}	14.6^{i}	9.1^{c}	14.4^{e}	6.9^{g}	8.7^{h}	4.7^{d}	0.0^{de}	-1.3^{b}	0.7^{de}	7.2^{cd}	19.8^{d}	15.8^{d}
$P_2 \times P_5$		-7.6^{ab}	-3.4^{ab}	-2.6^{a}	-1.7^{b}	2.9^{b}	-4.3^{a}	-2.1^{c}	-1.8^{b}	1.8^{c}	-1.7^{bc}	-2.3^{b}	0.2^{d}	19.0^{f}	30.5^{g}	27.3^{f}
$P_3 \times P_4$		-0.2^{ef}	7.6^{f}	5.4^{d}	13.2^{h}	27.8^{j}	20.6^{f}	-2.7^{bc}	5.2^{f}	1.8^{c}	-0.4^{cd}	2.2^{d}	1.3^{de}	3.8^{a}	9.1^{b}	1.6^{a}
$P_3 \times P_5$		-1.5^{de}	1.5^{d}	0.2^{b}	5.3^{d}	14.1^{d}	12.5^{d}	-0.8^{d}	5.6^{g}	5.1^{d}	-2.1^{b}	0.4^{c}	-1.1^{c}	6.2^{c}	13.4^{c}	32.2^{g}
$P_4 \times P_5$		-0.8^{e}	5.7^{e}	4.6^{d}	-2.8^{a}	1.8^{a}	-4.2^{a}	-3.5^{b}	-1.8^{b}	-4.5^{b}	-2.5^{b}	-2.5^{b}	-3.7^{b}	13.8^{e}	26.5^{f}	13.1^{c}
LSD	0.05	1.624	1.262	1.256	0.130	0.152	0.152	1.075	1.129	1.151	1.455	1.176	1.111	1.078	0.984	1.059
	0.01	2.190	1.703	1.694	0.175	0.205	0.205	1.450	1.523	1.553	1.963	1.587	1.499	1.454	1.328	1.429
P-value		0.0021	0.0001	0.0074	0.0075	0.0075	0.0075	0.0089	0.0068	0.0008	0.0018	0.0051	0.0016	0.0048	0.0088	0.0004

The means with the same letter indicate non significant differences, while the means with different letters indicate significant difference.

Table 4 Heterosis value (%) over Mid-parents in yield and quality traits of the 10 F_1 hybrids of Faba bean in 3 levels of inbreeding (S_0, S_1, and S_2).

Crosses		Heterosis (%) over Mid-parents																	
		Total dry seed yield			No. of pods/plant			Weight of 100-seeds			Shellout percentage			Pod filling			Protein Content		
		S_0	**S_1**	**S_2**	**S_0**	**S_1**	**S_2**	**S_0**	**S_1**	**S_2**	**S_0**	**S_1**	**S_2**	**S_0**	**S_1**	**S_2**	**S_0**	**S_1**	**S_2**
$P_1 \times P_2$		114.4^{e}	128.8^{e}	115.7^{j}	39.4^{h}	38.9^{g}	41.0^{h}	-2.8^{cd}	-0.2^{f}	-2.4^{e}	8.9^{f}	11.1^{f}	10.7^{g}	34.5^{j}	13.2^{g}	25.7^{j}	-8.5^{a}	-7.2^{c}	-4.1^{ef}
$P_1 \times P_3$		91.3^{de}	105.3^{de}	102.7^{i}	27.0^{f}	26.8^{d}	24.4^{c}	-3.3^{bc}	-0.8^{e}	-2.4^{e}	9.3^{f}	7.0^{e}	5.8^{e}	0.4^{b}	-4.1^{c}	-4.1^{c}	-1.9^{e}	-7.6^{c}	-3.3^{f}
$P_1 \times P_4$		86.2^{cde}	87.2^{cd}	86.8^{h}	16.5^{b}	13.3^{a}	12.5^{a}	-6.0^{a}	-4.0^{b}	-2.9^{d}	-4.7^{c}	-2.2^{bc}	-0.2^{c}	21.2^{h}	18.9^{j}	22.2^{i}	-3.4^{d}	2.9^{f}	-4.5^{ef}
$P_1 \times P_5$		75.1^{cde}	71.1^{bc}	77.8^{g}	38.5^{g}	49.0^{j}	36.8^{f}	-0.5^{g}	-2.5^{d}	-1.0^{f}	7.6^{e}	7.2^{e}	6.8^{f}	22.9^{i}	12.6^{f}	14.8^{g}	-4.4^{cd}	-15.3^{a}	-8.5^{c}
$P_2 \times P_3$		23.0^{a}	45.3^{ab}	32.0^{b}	15.8^{a}	20.4^{b}	21.1^{b}	-4.1^{b}	-0.6^{ef}	-5.0^{b}	-8.1^{a}	-6.3^{a}	-7.5^{a}	19.7^{g}	4.5^{d}	6.4^{f}	-5.5^{c}	-0.3^{e}	-6.5^{d}
$P_2 \times P_4$		85.8^{cde}	82.6^{cd}	66.2^{f}	25.4^{e}	26.4^{c}	24.6^{c}	-3.1^{cd}	-5.4^{a}	-7.1^{a}	9.3^{f}	16.4^{h}	18.6^{i}	16.6^{f}	18.8^{i}	21.2^{h}	6.8^{g}	6.5^{h}	-3.1^{f}
$P_2 \times P_5$		53.1^{acd}	43.5^{ab}	39.0^{d}	41.6^{j}	39.8^{h}	39.4^{g}	-1.5^{ef}	-3.4^{c}	-4.8^{b}	-6.3^{b}	-2.9^{b}	-3.3^{b}	8.2^{d}	18.4^{h}	5.5^{e}	-6.8^{b}	-8.7^{b}	-8.7^{c}
$P_3 \times P_4$		21.4^{a}	27.1^{a}	19.2^{a}	39.9^{i}	48.1^{i}	49.4^{i}	-1.0^{fg}	1.2^{h}	-2.1^{e}	-4.1^{c}	-1.6^{c}	-0.5^{c}	0.8^{c}	-8.2^{a}	-7.9^{b}	-9.0^{a}	-7.4^{c}	-12.0^{b}
$P_3 \times P_5$		46.0ac	57.7abc	46.5^{e}	22.7^{c}	30.5^{f}	27.2^{d}	-2.3^{de}	0.4^{g}	-2.0^{e}	2.5^{d}	2.2^{d}	0.9^{d}	-0.6^{a}	-6.4^{b}	-10.4^{a}	3.5^{f}	5.3^{g}	-4.9^{e}
$P_4 \times P_5$		48.1^{ac}	39.6ab	38.3^{c}	24.0^{d}	29.5^{e}	29.5^{e}	-2.8^{cd}	-4.9^{a}	-4.2^{c}	9.3^{f}	13.9g	16.0^{h}	12.0^{e}	5.5^{e}	0.0^{d}	-3.8^{d}	-4.4^{d}	-15.6^{a}
LSD	0.05	**42.882**	**33.196**	**6.051**	**0.358**	**0.267**	**0.389**	**0.988**	**0.516**	**0.404**	**0.834**	**0.705**	**0.676**	**0.046**	**0.014**	**0.014**	**1.197**	**1.056**	**1.281**
	0.01	**57.853**	**44.785**	**8.164**	**0.483**	**0.360**	**0.524**	**1.332**	**0.696**	**0.546**	**1.126**	**0.951**	**0.912**	**0.062**	**0.020**	**0.020**	**1.615**	**1.425**	**1.729**
P-value		**0.0063**	**0.0019**	**0.0023**	**0.0017**	**0.0063**	**0.0055**	**0.0001**	**0.0012**	**0.0067**	**0.0053**	**0.0000**	**0.0009**	**0.0085**	**0.0030**	**0.0064**	**0.0007**	**0.0003**	**0.00005**

The means with the same letter indicate non significant differences, while the means with different letters indicate significant difference.

and S_2 levels. The heterosis values for this trait differed across the generation levels, for instance it ranged from −8.1 to +9.3 in the S_0 generation.

Significant mid-parent heterosis for greater pod filling (%) was detected in all crosses, except the cross P3 × P5 which showed significant negative heterosis in the three levels of inbreeding, whereas P3 × P4 and P1 × P3 showed significant negative heterosis in hybrids derived from both the S_1 and S_2 generations. Only one cross (P1 × P4) at S_1 level and two crosses (P2 × P4 and P3 × P5) at both S_0 and S_1 levels showed a significant positive increase in protein content percentage. On the other hand, significant negative heterosis was noticed in all levels of crosses for P1 × P2, P1 × P3, P1 × P5, P2 × P3, P2 × P5, P3 × P4 and P4 × P5. Abd El-Aziz [36] found significant estimates for heterosis and inbreeding depression for most of the studied traits in most crosses in F_2 generation. Bargale and Billore [32] studied 21 F_1 and F_2 faba bean hybrids and concluded that parental diversity was not associated with greater heterosis. High heterosis was found to be coupled with high inbreeding depression in a number of cross-combinations for yield and some yield components.

In this study mid-parent heterosis values (%) were estimated for all traits of the 10 F_1-hybrids at the 3 levels of inbreeding. For most characters some hybrids showed significant positive heterosis over mid-parent value. These results were in accordance with those of many investigators such as Ibrahim [5] who found several crosses recorded significant positive heterosis percentages relative to mid parent and better parent for seed yield per plant and 100-seed weight ranging from 17.46–84.95% and, 8.53–23.26% relative to mid-parent, respectively. Obiadalla-Ali et al. [6] stated that, the majority of crosses exhibited significant better parents heterosis estimates for all studied traits.

On the other hand, some crosses in our investigation, showed significant negative values of heterosis i.e. heterosis depression. Some hybrids in faba bean show negative heterosis for some traits [5,9,37,38]. Additive gene action was predominant for these traits. Significant effect for several traits such as number of branches per plant, pod setting percentage, number of pods per plant, 100-seed weight, shellout percentage and pod filling percentage [34,39]. These heterotic effects may range from significantly positive to significantly negative for various traits according to genetic makeup of the parents. Heterotic effects over mid and better parents were detected in most crosses by EL-Harty et al. [28]. Positive and significant heterosis percentages over mid-parents or better parent were reported for faba bean characters which varied according to the cross combinations and traits [38,39], Generally, high SCA effects in faba bean for yield and related traits were associated with genetic diversity of parents.

There was a wide range in level of heterosis value over the mid-parent in respect of the level of hybrid vigor (Table 4) obtained in the studied traits. The highest values of heterosis over the mid-parent occurred for total dry seed (128.8%) followed by No. of pods/plant (49.4%), pod filling percentage (34.5%), pod setting percentage (34.0%), No. of branches/plant (24.7%), shellout percentage (18.6%), plant height (8.8%) and protein content percentage (6.8%). The lowest value of heterosis was shown by weight of 100-seeds (1.2%).

The highest values of heterosis were generally obtained when P2 (Giza 843) was included in the cross, so it can be concluded that the genotype P2 can be considered the best general combiner for most traits. Moreover, it was also found that specific combinations among the studied parents gave the highest heterosis values over mid-parent. For example cross (P1 × P2) was the best cross for total dry seed yield and pod filling percentage, cross (P2 × P3) for No. of branches/plant and pod setting percentage, cross (P2 × P4) for shellout percentage and protein content percentage and cross (P3 × P4) for No. of pods/plant. The frequency and level of heterosis were related more to SCA than to the genetic divergence of the parents in faba bean [5,6,28,39].

Level of polymorphism

Twelve out of 20 arbitrary primers revealed genetic polymorphism among the five parental genotypes (Table 5). A total of 65 amplification products were scored polymorphic (Fig. 1 and Table 5). The percentage of polymorphic bands detected ranged from 33% (OPA-13) to 100% (OPG-09) with an average of 66.47% (Table 5). The range of polymorphic bands was

Table 5 Primers used in RAPD analysis, total number of fragments detected by each primer and polymorphism among five parental faba bean genotypes.

Primer Name		Primer sequence (5′ → 3′)	Amplified bands		Polymorphic bands (%)	Fragments size base pair	
			Fragments number	Polymorphic bands		Larger	Smaller
1	OPAM-01	TCACGTACGG	9	8	88.9	687	188
2	OPA-17	GACCGCTTGT	12	9	75.0	1150	97
3	OPG-09	CTGACGTCAC	8	8	100	1018	435
4	OPP-05	CCCCGGTAAC	11	6	54.5	1088	230
5	OPH-01	GGTCGGAGAA	9	7	78.0	1300	325
6	OPAW-10	GTTGTTTGCC	5	2	40.0	720	202
7	OPAD-06	AAGTGCACGG	9	6	66.7	1237	178
8	OPAT-08	TCCTCGTGGG	4	2	50.0	555	245
9	OPW-13	CACAGCGACA	7	5	71.0	915	200
10	OPA-13	CAGCACCCAC	3	1	33.0	573	255
11	OPAR-05	CATACCTGCC	10	9	90.0	1228	160
12	OPF-20	GGTCGGAGAA	4	2	50.0	900	315
Total			91	65			
Mean			7.58	5.42	66.47%		

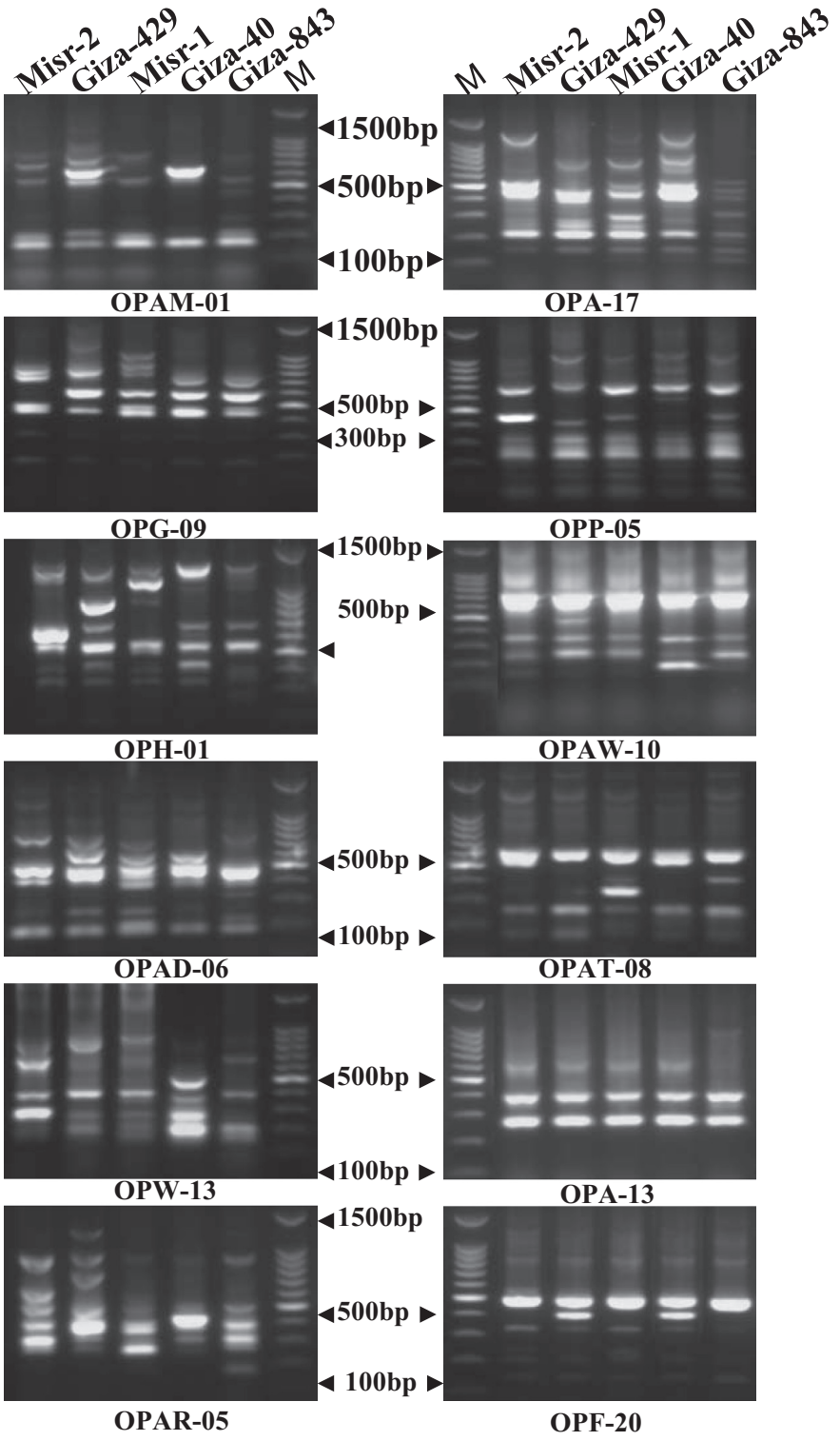

Fig. 1 RAPD profiles obtained for five parental faba bean genotypes amplified with 12 primers and M = 100 bp ladder size marker.

1 to 9 with an average of 5.42 per primer. Similar results of level of polymorphism were obtained using different DNA markers such as: RFLP (61.9%) [40]; RAPD (76.6%) [17]; SSR (72%) [41] and SSAP (71%) [42]. The level of polymorphism obtained in this study was smaller than 86.90% obtained by Alghamdi [43] using RAPD markers. The overall numbers of amplified bands per primer were in agreement with those obtained by Abdel Sattar and El-Mouhamady [44] but smaller than those obtained by Tanttawi et al. [17], who reported a range from 3 to 21 bands with an average of 11.8 bands. The fragments sizes obtained were from 97 (OPA-17) to 1300 bp (OPH-01) (Table 5). Similar results were obtained

by El-Sayed et al. [18], applying RAPD markers on Egyptian faba bean.

Dendrogram analysis

Genetic relationships based on RAPD markers revealed that the genetic similarities among faba bean genotypes ranged from 0.61 (Giza 40 and Giza 843) to 0.77 (Misr 1 and Misr 2) (Table 6). The genetic similarity values ranged from 0.55 to 0.83 among 6 different varieties using RAPD markers [17]. Zeid [45] reported similar values, ranging from 0.53 to 0.88 among 79 inbred lines of recent elite faba bean using ALFP markers. The five parental genotypes separated into three clusters (Fig. 2). The first cluster contained Misr 1 and Misr 2 at a relatively high level of similarity of 0.77. Giza 40 and Giza 429 clustered at 0.75 level of similarity on the second cluster. Giza 843 was alone in the third cluster which clustered at 0.66 level of similarity with the other genotypes in this study.

The Euclidean distance, based on the means of quantitative traits was calculated to establish the relationship among genotypes. The range of Euclidean distance among the genotypes was relatively wide from 18.54 (Misr 2 and Giza 429) to 233.44 (Misr 1 and Giza 40) (Table 7). Our result indicated that the amount of phenotypic variation among these parental lines was relatively high and reflects the genetic diversity of the genes controlling these characters. The five genotypes divided into two distinct clusters. Bootstrap values (Fig. 3) showed a pattern of high genetic variation, where Misr 1 was in the first cluster separated from the other genotypes at a wide Euclidean distance of 169.57. The second cluster sub-divided into three sub-clusters, the first sub-cluster included Misr 2 and Giza 429, which separated at relatively low Euclidean distance of 18.54. The second sub-cluster contained Giza 843 which clustered at 53.48 with the first sub-cluster, and Giza 40 was alone in the third sub-cluster.

Table 6 Similarity matrix (%) for five parental faba bean genotypes according to Nei and Li's coefficient obtained from 91 RAPD bands.

Genotypes	Misr 2	Giza 429	Misr 1	Giza 40	Giza 843
Misr 2	1.00				
Giza 429	0.70	1.00			
Misr 1	0.77	0.72	1.00		
Giza 40	0.71	0.75	0.69	1.00	
Giza 843	0.72	0.61	0.69	0.61	1.00

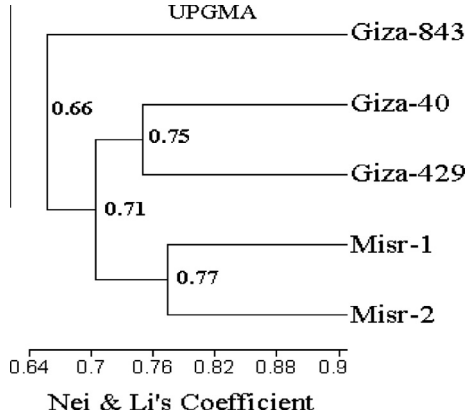

Fig. 2 Dendrogram generated by UPGMA cluster analysis according to Nei and Li's coefficient using 91 RAPD bands among five parental faba bean genotypes.

Table 7 Euclidean distance matrix of five parental faba bean genotypes using means of all studied characters.

Genotypes	Misr 2	Giza 429	Misr 1	Giza 40	Giza 843
Misr 2	0.00				
Giza 429	18.54	0.00			
Misr 1	156.58	172.19	0.00		
Giza 40	78.35	61.73	233.44	0.00	
Giza 843	47.54	60.14	116.07	120.79	0.00

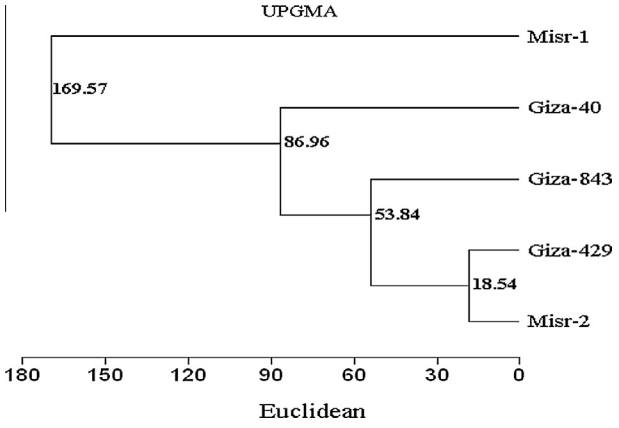

Fig. 3 Dendrogram based on UPGMA cluster analysis showing the Euclidean distances among five parental faba bean genotypes using means of all studied characters.

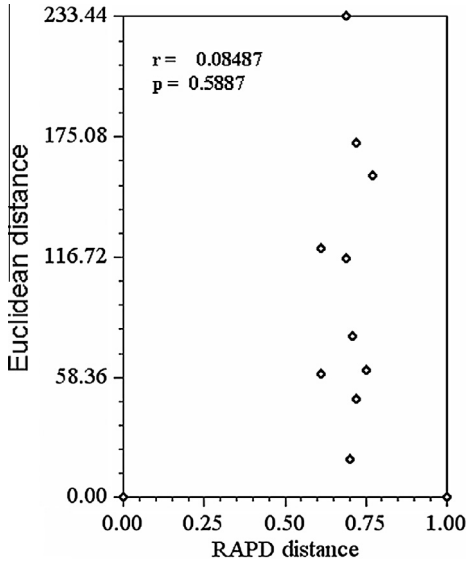

Fig. 4 Correlation between Euclidean distance and RAPD distance methods generated by NTSYS-pc Ver. 2.1 program.

The correlation between Euclidean distance and RAPD distance was not significant $r = (0.085)$ (Fig. 4). A negative correlation of −0.40 between Euclidean and RAPD distances was obtained by Tanttawi et al. [17]. The observed relationships using molecular markers may provide information on the history and biology of cultivars but it does not necessarily reflect what may be observed with respect to agronomic traits [46]. Genetic markers such as RAPDs may accurately assay the degree of genetic change between two genomes, but they may not necessarily reflect the divergence in terms of changes in traits of agronomic importance.

Conclusions

From the data presented in this investigation, it can be concluded that improvement of most traits of faba bean could be achieved by hybridization among the studied parental genotypes. While some specific combinations among these parents produced the highest values of heterosis over mid-parent, P2 (Giza 429) can be considered to be the best general combiner for most traits.

Some traits of faba bean showed some inbreeding depression after the first cycle of selfing (S_1) whereas no further, decrease was found at the S_2 generation. This indicates that stability of the genetic constituent of these parental genotypes could be achieved after one selfing generation. Therefore, hybridization among these parents at the S_1 or S_2 generations is recommended. Hybrid progeny of stable parents exhibited stability for its traits. RAPD markers and agronomic characterization will be useful tools for assessing the genetic diversity, and understanding the breeding patterns of faba bean.

Conflict of Interest

The authors have declared no conflict of interest.

Compliance with Ethics Requirements

This article does not contain any studies with human or animal subjects.

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