African Journal of Agricultural Research Vol. 6(30), pp. 6387-6390, 12 December, 2011
Available online at http://www.academicjournals.org/AJAR
DOI: 10.5897/AJAR11.762
ISSN 1991-637X ©2011 Academic Journals

Full Length Research Paper

Analysis of the degree of cross-fertilization and auto-fertility and their impact on breeding faba bean (*Vicia faba* L.) cultivars grown in Sudan

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Accepted 22 July, 2011

In faba bean, the degree of cross-fertilization and auto-fertility is a crucial character in any breeding program. The degree of cross- and auto-fertility of four faba cultivars were assessed at three locations in Sudan, using hilum colour as a morphological marker. The variation between the cultivars and between locations for the degree of cross-fertilization was extremely small, which ranged from 10.6 to 12.7%. The degree of auto-fertility was high in the four cultivars, reaching up to 88%, therefore, line cultivar could be produced. As line cultivar has a risk of low yield stability, the search for plant traits that attract pollinating insects could contribute to increase cross-fertilization and as a result heterosis could be used for the improvement of cultivars. Otherwise, the floral biology of the bean plant should be manipulated towards full autogamy.

Key words: *Vicia faba* L., breeding, cross-fertilization, auto-fertility, line cultivar, bee pollinator.

INTRODUCTION

Faba bean (*Vicia faba* L.) is an insect cross-pollinated species with a genuine mixed mating system. One fraction of the seed accrues from self-fertilization; the remnant part from cross-fertilization. The crop is a main source of edible protein for food and feed. In addition, it contributes to soil fertility through nitrogen fixation and minimizing the impact of chemical fertilizers on environment (Palmer et al., 2009).

Faba bean is the most important pulse legume crop in Sudan. It is grown as an irrigated winter crop mainly in the northern part of Sudan (Northern and River Nile states and, to limited extent, Khartoum) where environmental conditions suit its agro-ecological needs. The crop is occupying 60% of pulse acreage followed by haricot beans (*Phaseolus vulgaris* L.), chick pea (*Cicer arietinum*) and lentils (*Lens culinaris*). The production and consumption of faba bean have been steadily rising during the last two decades as the crop had prevailed its important as the main component of the Sudanese diet. As diet, it constitutes to the main dish on the breakfast and dinner of the large sector of the population, especially low income group in the urban areas. In some years the supply falls short of demand and therefore quite an amount is imported from abroad. This low supply situation stems from the limited agricultural resources in the traditional areas and low yield obtained by the farmers. Therefore, the main avenues for solving such supply position are through the vertical improvement of production in the traditional areas of production and/or through horizontal expansion in the new areas. Recently, efforts were made to extend its production into other non-traditional warmer areas, that is, Gezira, New Halfa and Rahad Schemes, where land and water are available. The productivity of the crop is low (2860 kg/ha) compared to the productivity in Egypt (3267 kg/ha) (AOAD, 2005), where it is also grown by irrigation.

One of the major constraints that limit the realization of full yield potential of faba bean and cause instability in yield is thought to be the partial dependence of the crop on insects for pollination. Pollinators help to increase the rate of fertilized ovules, both through self- and cross-pollination and thus ultimate seed set (Richards, 2001). The average degree of cross-fertilization was reported to

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Table 1. Mean degree of cross-fertilization of four faba bean cultivars grown at three locations (Dongla, Shambat and Hudeiba) in Sudan.

Location/cultivar	Number of tested plants		Degree of cross-fertilization (%)			
	Mother plants	progeny	Min.	Max.	Mean	±Sd
Dongla						
Hudeiba/93	40	1189	5.0	21.4	11.95	4.66
Basabier (BB7)	52	1663	5.0	22.6	10.64	4.51
Edammar	40	1221	5.3	21.4	10.96	4.10
Sulaim (SLM)	43	1323	5.0	23.5	11.05	5.12
Mean	43.8	1349	5.1	22.24	10.90	4.60
Hudaiba						
Hudeiba/93	44	1126	6.45	23.33	12.15	5.05
Basabier (BB7)	42	1158	6.45	20.69	10.60	4.70
Ed-Dammar	42	1103	5.26	20.69	11.40	4.61
Selaim (SLM)	39	1064	6.45	22.22	11.87	4.79
Mean		1112.75	6.15	21.73	11.50	4.79
Shambat						
Hudeiba/93	45	1290	5.56	25.00	12.00	4.92
Basabier (BB7)	37	1167	5.13	20.83	12.71	4.60
Ed-Dammar	35	1078	5.00	25.00	11.11	5.05
Selaim (SLM)	40	1116	5.00	23.33	11.59	5.14
Mean	39.25	1162.75	5.17	23.54	11.85	4.93

*Sd= Standard deviation.

range from 12 to 18% at Hudeiba (Salih, 1987) and from 14.2 to 17.4% at Shambat (Salih et al., 1994). At present, seed stock of the previous known cultivars was subjected to great mechanical and genetic mixing with the stock of other faba bean populations, therefore, an obvious loss of the initial agronomic and genetic features of the cultivars took place; however, substantial information on these cultivars has bean generated from the previous genuine research efforts.

To improve both yield and yield stability of the present Sudanese faba bean cultivars, information about their reproductive mode is essential. The objective of this study was to estimate the degree of auto-fertility and cross-pollination and their effect in improving the present cultivars.

MATERIALS AND METHODS

To achieve the above-mentioned objectives, a field survey carried out and seeds were collected from faba bean fields in Dongla, Hudaiba (Northern State), Hudeiba (River Nile State) and Shambat (Khartoum State) during the harvest time in 2006/2007 season. Seeds were collected on the basis of hilum color. Five seeds from about 500 white hilum plants and 200 black hilum plants from each of the known cultivars grown in these areas, that is, Selaim, Hudeiba/93, Bassbier and Ed-Damar) were harvested individually. From the collected seeds, three experiments (Experiments 1, 2 and 3) were conducted.

Experiment 1

In this experiment, the degree of auto-fertility was assessed by taking the white and black hilum seeds of each of the four cultivars from the three locations and sown at Shambat in bee-proof isolation cages, covering an area of 60 m^2 each. Three generations, S_0 (original seed), S_1 (once-selfed) and $S_{2'}$ (twice-selfed) were derived and evaluated in 2007/2008, 2008/2008 and 2009/2010 seasons (Table 1) for their degree of auto-fertility. Auto-fertility was estimated according to Link (1990).

In this experiment, 8 plants were grown per plot in two replications. Each plant produced 3 to 5 flowers per inflorescence. In this experiment we either reduced the number of the first 8 flowering nodes to 2 per inflorescence (other inflorescence and tillers were removed), resulting in a total of 16 flowers per plant or left the inflorescence intact. All flowers were left untripped. The assumption is that all fertilized flowers develop pods (Link, 1990). The rate of fertilization was calculated as (number of seed containing pods/16) × 100, in this way, the relative proportion of flowers that self-fertilized without being tripped were scored, thus measuring the degree of auto-fertility (Drayner, 1959). In plants where all inflorescence were left intact, the number of pods/plant, number of seeds/pod, 100-seed weight and seed yield/plant were rcored for the generations in each cultivar. This way, the effect of selfing on the performance of the cultivars in these traits will be assessed, which also indicate degree of auto fertility, otherwise, the cultivars show reduction performance due the effect of inbreeding.

Experiment 2

This experiment was carried out to estimate the degree of cross-

Table 2. Mean degree of autofertility in three generations (S_o), once-selfed (S_1) and twice-selfed (S_2) of four faba bean cultivars at Shambat.

Cultivar	Auto-fertility/Generation			Mean	LSD (0.05)
	S_0	S_1	S_2		
Hudeiba/93	0.92	0.87	0.85	0.88	0.08
Bassbeir	0.88	0.86	0.84	0.86	0.06
Ed-Damar	0.90	0.89	0.85	0.88	0.06
Selaim	0.89	0.86	0.85	0.86	0.07
Mean	0.90	0.87	0.85	0.87	
SE±	0.02	0.03	0.01	0.03	

fertilization. Scoring for cross-fertilization was based on a morphological hilum colour, which is a monogenic trait and black is fully dominant. It controlled by the genotype of the mother plant**,** hence, seeds harvested from a plant are uniform as to their hilum colour. The white and black hilum seeds of each cultivar were bulked in a ratio of 1 white to 3 black and sown in 2007/2008 season at Dongla, Hudeiba, and Shambat. At maturity, only white hilum seed were harvested at the three locations and sown in 2008/2009 at Shamabt to estimate the degree of cross-fertilization that occurred at the three locations. The degree of cross-fertilization was estimated according to Metz et al. (1994) as frequency of heterozygotes in the progeny of homozygous mother plants as follows:

$$\text{Degree of cross-fertilization} = \frac{\text{Number of black helium seed progeny}}{\text{Total number of scored progeny}}$$

All heterozygous descendents had originated from cross-fertilization, because homozygous recessive mother plants only carry the recessive allele.

RESULTS

Table 1 shows the mean percentage of cross-fertilization of the four faba bean cultivars at each of the three locations (Dongla, Hudeiba and Shambat). In general, the variation between cultivars and between locations for the degree of cross-fertilization was extremely small. The estimates of the degree of cross-fertilization ranged from 5 to 23% for the individual plant in each cultivar at the three locations with an average ranging from 10.6 to 12.7% for both cultivars and locations. At Dongla, the estimate of the percentage of cross-fertilization for the individual plant in each cultivar ranged from 5.0 to 22.2% with an average of 10.9%. At Hudeiba, the range was from 6.2 to 21.7% with an average of 11.5%, whereas at Shambat, the range was from 5.2 to 11.9% with the average of 23.5%.

The degree of auto-fertility for the generation of each cultivar was presented in Table 2. Generally, the mean values of the degree of auto-fertility of the individual cultivar reached up to 88% as Hudeiba/93 and the variation between the generations of the cultivars was not significant. The range was from 0.84 (for Bassabier-S2) to 0.92 (for Hudeiba/93-S0). The overall degree of auto-fertility for the four cultivars was on average of 0.90 for S_0, 0.87 for S_1 and 0.85 for S_2 with an average of 87%. The high degree of auto-fertility was also shown by the similar values in number of pods/plant, number of seeds/pod, 100-seed weight and Seed yield/plant for S_0 outside the cage and for S_1 and S2 generations of the four cultivars inside the cages (Table 3).

DISCUSSION

The low estimate of the degree of cross-fertilization of the present material was in accord with that of Salih (1987) who reported nearly similar degree of cross-fertilization (12 to 18%) at Hudeiba and that of Salih et al. (1994) who reported on the degree of cross-fertilization at Shambat (14.2 to 17.4%). These results disagree with that of Suso et al. (2001) and Gasim et al. (2004) who reported the effect of genotype and environment on the degree of cross-fertilization. This low estimate of the degree of cross-fertilization of this material may be due to poor activities of pollinators. In addition, as the bee often visits several flowers on the same plant in succession, the pollen which it brings to a flower is not necessarily 100% foreign (Drayner, 1959).

The high degree of autofertility of the four cultivars may due to that these bean cultivars behave predominantly like self-fertilizer, since there was no reduction in the performance of the three there generations of the cultivars in the traits of number of pods/plant, number of seeds/pod, 100-seed weight and Seed yield/plant. Due to the high autofertility in these cultivars, the low estimate of the degree of cross-fertilization may not be a disadvantage as line cultivars could be developed from this material. The line cultivar offers an opportunity to maximize selection intensity and makes breeding easier, in addition it is associated with lowering inbreeding depression and increasing autofertility (Link, 1990), hence ensured seed production in the absence or poor activity of insect pollinators (Drayner, 1959).

Although line cultivars could be recommended as breeding strategy for the present cultivars, line breeding is not the appropriate method for the improvement of yield and yield stability. Breeding as line cultivar leaves

Table 3. Mean performance of some yield traits in three generations (S0, S1 and S2) of four faba bean cultivars at Shambat.

Cultivar/trait	Hudeiba/93				Bassabier				Ed-Damar				Selaim				LSD
	S0	S1	S2	Mean	S0	S1	S2	Mean	S0	S1	S2	Mean	S0	S1	S2	Mean	
No. of pods/plant	16.7	16.1	15.7	16.0	15.6	15.0	15.0	15.2	16.4	16.3	16.1	16.3	13.9	13.5	13.5	13.6	0.53
No. of seeds/pod	2.4	2.3	2.3	2.3	2.5	2.4	2.4	2.4	2.5	2.3	2.4	2.4	2.2	2.3	2.3	2.3	0.11
100-seed weight (g)	43.5	43.5	43.7	43.6	43.7	43.6	43.7	43.7	45.3	45.2	54.2	45.2	66.3	66.0	65.9	66.1	0.30
Seed yield/plant (g)	15.9	15.8	15.9	15.9	15.5	15.5	15.4	15.4	16.1	16.1	16.1	16.1	15.2	15.1	15.1	15.1	0.34

heterosis unexploited, moreover, there is a risk of low yield stability (Stelling et al., 1994). To improve the yield of the present cultivars, pollinators are particularly important, because their activity determines both the incidence of ovule fertilization and its genetic character (self-versus cross-fertilization) as reported by Free (1993) and Bond and Kirby (2001).

Therefore, search for plant traits that attract pollinators and thus contribute to cross-fertilization deserves to be investigated. Plants with attractive floral traits were reported to enhance pollinator conservation as well as yield and yield stability (Palmer et al., 2009). That means it may be worthwhile to check which insects (solitary or social bees) could be the relatively best pollinator to our faba bean cultivars in order to multiply and breed them as well as to improve their foraging places and nesting sites. This way the degree of cross-fertilization could be increased and heterosis could be used for improvement of cultivars (e.g. synthetic cultivars). Otherwise, the floral biology of the bean plant should be manipulated towards full autogamy and thus handling the cultivars may be improved and maintained by the conventional breeding methods.

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