Efficiency of different breeding strategies in improving the faba bean productivity for sustainable agriculture

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Abstract

Breeding methods applied to faba bean are either based on self pollination by developing lines under insect proof cages or baed on open pollination by developing lines under natural pollination conditions. The purpose of this research is to compare the performance of pedigree selection method (PSM) as a self pollinated breeding method and recurrent selection methods (RSM) and synthetics (SYN) as an open pollinated method). Eleven diverse accessions were used to develop in F6 generation 24 lines using PSM, 35 lines using RSM and nine synthetics from 2012 to 2016. The different developed lines were evaluated in two winter cropping seasons (2014/2015) and (2016/2017) in an alpha design with two replications. Significant differences among lines and among breeding methods were obtained for biological and grain yield, days to flowering, days to maturity, branches per plant, pods per plant, hundred seed weight. Synthetic populations had higher yield than RSM lines; whereas RSM lines revealed higher yield performance than PSM lines. Multivariate analysis indicates that most of the variation among different lines developed by different methods is due to variation in number of branches, days to flowering, biological and grain yield. Open pollinated cultivars were found to be more appropriate to increase the yield in farmers' fields and may play a critical role in conserving wild pollinators, but ensuring optimal yields might require capacity building for farmers concerning wild pollinators.

Key words: faba bean, breeding methods, recurrent selection, pedigree method, synthetic cultivars.

Introduction

Faba bean (*Vicia faba* L.) cultivation can be traced back to the beginning of agriculture (Cubero 1973). It is one of the oldest crops grown in the Fertile Crescent (Caracuta et al. 2015). Presently, faba bean is one of the most important grain legumes in East Asia, East and North Africa and the Middle East, and is classified as the fourth most widely grown cool season legume (FAOSTAT 2019). The crop is grown across a wide agro-geographical region (Bond et al. 1985) in more than 38 different diversified cropping systems. It remains an important crop because of its high-yield potential, nutrition-dense grains, high protein content and role as a forage crop (Burstin et al. 2011). The major constraints of this crop are yield instability due to several diseases (Maalouf et al. 2013; Maalouf et al. 2016) and various abiotic stresses e.g. drought and heat or cold during flowering (Maalouf et al. 2015).

Faba bean is a partial allogamous crop and is an entomophilous species, it requires insect pollinators to ensure appropriate production. Though the pollination impact of faba bean has been identified as only "modest" (production reduced by 10 - <40% in case of absence of pollinators, Klein et al. 2007), pollination can highly affect yields of faba bean (Aouar-sadli et al. 2008; Nayak et al. 2014; Andersson et al. 2014) and even balance negative effects of heat stress (Bishop et al. 2016). Pollinator decline (Biesmeijer et a. 2006; Potts et al. 2016; Hallmann et al. 2017) can cause collapse of plant-pollinator networks (Lever et al. 2014) and affects not only agriculture, but all ecosystem services to a high extent and can cause simultaneous interlinked poverty spirals (Christmann 2019b). Pollinator protection is crucial for humankind (Christmann 2019 a, b; Potts et al. 2016). Egan et al. (2018) warned that breeding can negatively affect the attractivity of crops for pollinators and the health of pollinators, they suggested these potential impacts should be studied more. Bailes et al. (2018) analyzed different sugar concentrations and the force needed to open the flower of faba bean. This study can widen the spectrum of breeders' research. The color of petals is also a main determinant to make a flower attractive for pollinators, Miguel‑Peñaloza et al. (2019) showed the impacts of color on the example of another *Fabacaea* (*Desmodium grahamii).*

Currently, faba bean is pollinated mainly by wild bees, e.g. carpenter bees, bumblebees *(Bombus lapidarius, B.pascuorum, B. hortorum),* mason bees, longhorn bees, digger bees (*Anthophora plumipes), leafcutter bees* and hoverflies; honeybees harvest faba bean nectar as well, but can face problems to open the flower and pollinate effectively (see also Marzinzig et al. 2018; Aouar-sadli et al. 2008; Klein et al. 2007). Marzinzig et al. (2018) highlighted the importance of specialized wild pollinators with long tongue like *B. hortorum* as effective pollinators of faba bean, but the wide range of species promotes faba bean pollination also in regions, where specific species are not available (e.g. bumblebees prefer moderate temperature and do not live in Ethiopia, which is an important producer country).

However, are current fields – research sites and agricultural landscapes managed by farmers – attractive for wild pollinators or can the fields be improved on large scale? Wildflower strips (WFS), the most common approach for pollinator conservation in agricultural lands, are not favored by farmers even if a compensation is paid (Kleijn et al. 2019). As WFS require external funds to motivate farmers, they are not an option for Low and Middle Income Countries (LIC, MIC) producing faba bean on large scale like Ethiopia or India. Protection on large scale is only possible in LIC and MIC, if the habitat enhancement creates a win-win-situation for farmers and the environment (Christmann and Aw-Hassan 2012; Christmann et al. 2017; Christmann et al. in review).

The TEEB-based Farming with Alternative Pollinators (FAP) approach (Christmann et al. in review; Christmann 2019a; Christmann et al. 2017; Christmann and Aw-Hassan 2012) does not require rewards for farmers, as it induces higher net income per surface. FAP uses only marketable habitat enhancement plants (MHEPs) instead of weeds and includes nesting and water support out of local materials. Goulson et al. (2015) clearly pointed out, that pollinator decline is not only caused by lack of floral resources (addressed by WFS), but also by parasites and pesticides. Different to the WFS-approach, FAP has also an explicit focus on capacity building for farmers (Christmann et al. 2017; Christmann et al. in review), this is essential. The multidisciplinary FAP approach has potential for scalability even in LIC producing faba bean. Broad introduction of FAP-faba bean planting instructions might contribute to biodiversity protection in agricultural lands (Aichi target 7) and higher climate change resilience.

However, it requires broader collaboration between scientists, agricultural trainers and farmers than just providing enhanced seeds: capacity building concerning pollinator diversity, habitat requirements and sustainable field management. Whereas breeders of pollinator-independent crops can work far from farmers, in laboratories and stations, breeders and researchers working on pollinator dependent faba bean need to engage also in capacity building for farmers, because the knowledge of farmers on pollination and pollinators is low and agricultural practices can undermine pollination services (Christmann et al. in review; Aizen et al. 2019).

The major breeding purpose in faba bean is to increase yield and yield stability through combining different donors for resistance to biotic and abiotic stresses and increasing the level resistance or tolerance to biotic and abiotic stresses. As the value of the outcrossing rate varied from 4 to 84% (Bond and Pope 1974; Suso et al. 1999a, b), breeders tend to develop breeding lines using different breeding methodologies. The first breeding methods (Pedigree methods, Single Seed Descend) consist of developing pure lines with high level of autofertility. Several authors have proposed in their breeding programs to transform the mating system of faba beans towards autogamy (Kambal et al. 1976; Adcock and Lawes 1976; Bozzini and Chiaretti 1999) to develop inbred lines. These lines are uniform and are specifically adapted to organic farms (Ghaouti et al. 2008). However due to the partially allogamous nature of the crop and its susceptibility to inbreeding depression, it might not be easy to handle faba bean by the pedigree breeding method or any other breeding method used on self-pollinating crops (Lawes et al. 1983). The second approach is the application of recurrent breeding method as proposed by Hallauer (1981) and Rowland (1987). Hallauer (1981) suggested that recurrent selection would be a useful breeding method for preventing the loss of potentially desirable genes where the introduction of new germplasm and selection for adaptation are occurring at the same time. The third option is to develop synthetic varieties (Link et al. 1994a; Maalouf et al., 1999; Maalouf et al., 2002). Both recurrent and synthetic breeding methods may lead to exploit heterosis in faba bean cultivars and then to enhance yield and yield stability (Stelling et al., 1994, Link et al. 1994b, Abdelmula et al. 1999; Arbaoui and Link 2008), as well as to increase the resistance or tolerance to major abiotic (Gasim and Link 2007; Terzopoulos et al. 2008) and biotic stresses (Maalouf et al. 2008).

The purpose of this research is to evaluate the efficiency of three breeding methodologies (Recurrent selection, Pedigree selection and Synthetic cultivars) in improving sustainable yield, and study which traits can differentiate in the performance of different breeding methods

Materials and Methods

This study includes eleven faba bean accessions, which originated from Egypt, Morocco, China, United Kingdom and ICARDA (Table 1). These accessions were used to develop six crosses which were advanced into recurrent and pedigree lines in open pollination and under insect proof cages, respectively. The open pollination trials were conducted in open field to allow honeybees and high diversity of wild pollinators to provide services available near to the experimental station. The trials under insect proof cages was covered by tent to avoid bees to pollinate the breeding lines. A total of ten field experiments were conducted from 2012/2013 to 2016/2017 at ICARDA Terbol Research Station, Lebanon, Bekaa Valley (35.9 N, 33. 8E), altitude 890. These experiments are summarized as follows:

1- Pedigree and recurrent selection trials

(a) Two experiments were conducted in augmented design for 2013 summer season (June to October) to evaluate the performance of 150 F3 lines derived in open pollination (75 F3 lines) and in self-pollination (75F3 lines). Each line was planted in single row with 2 meters length and 10 seeds per line). Best performing single plants per line were selected in both conditions.

(b) Two experiments were conducted in augmented design for 2013/2014 winter season (November to June) to evaluate the performance of F4 single plant progenies selected in previous season. Lines selected in open field were evaluated in open fields to expose them to pollinators and lines selected under insect proof cages were planted under cages to avoid pollination. Each single plant was planted in single row (10 seeds per line). In self-pollination conditions, pedigree methods were applied for further selection while in open pollination recurrent selection was used and reserve seeds of each single plants were multiplied under cages.

(c) Two experiments were conducted in alpha lattice design for 2014 summer season for F5 single plants derived in open pollination and in self-pollination and planted in single row (10 seeds per line). In self-pollination conditions, pedigree methods were applied for further selection while in open pollination recurrent selection was used. 24 single rows selected under insect proof cages by pedigree method and 35 in open pollination using recurrent method in open field. For recurrent method, multiplied reserve seeds were used for further evaluation.

The intensity of selection varied from 1.37 to 1.52 from F3 to F5 generation in recurrent selection methods and from 1.24 to 1.84 for pedigree method (Table 2)

2- Synthetic development trials

(a) Topcross design, which includes the eleven parents and a tester with broad genetic base (mixture of all lines) was conducted in the winter of 2013/2014. Each parental line was planted 25 times randomly in single hills (3 plants per hill). One plant was covered to by insect proof cages during flowering time and another one left in open pollination conditions.

(b) The resulting progenies were evaluated during the summer of 2014 in open field and under screen houses to develop the synthetic varieties;

3- Evaluation trials

Two experiments were conducted in alpha design with two replications in two winter seasons (2014/2015 and 2016/2017) to compare the performance of the best 35 recurrent lines obtained in open field, 24 pedigree lines under screen houses, 9 synthetics and the 11 parental lines. The lines were planted in 2 rows with 4 meters length and 0.45 m distance and 10 cm between seeds. Data were recorded for overall plot. Syn_0 was evaluated 2014/2015 and Syn_2 in 2016/2017

4- Recorded data

The following data were recorded in all selected plants per plots for trials conducted from F3 to F5 and plot data were recorded in the evaluation trials in 205/2016 and in 2017/2018. The following recorded data was based on ontology described in Maalouf F (2018) a) the phenological traits recorded are : days to flowering (DFLR), days to maturity (DMAT), b) Grain yield components Grain yield kg/hectare (GY) and its components: Pods per plant (PNPLT), number of seeds per pod (SNP), number of seeds per plant (SNPLT), hundred seed weight (HSW), single-plant yield (GYPLT), c) Biological yield kg/ha (BY) and its components: Plant height (PLHT) and number of branches per plant (BRPLT)

5- Biometric analysis

Data from each augmented design experiment were examined for possible spatial variability in terms of a set of nine models. The best model was selected by using the method described by Singh et al. (2003). The spatial models accounted for the effects of complete randomized design (CRD), linear trends and first-order autocorrelations in the plot errors along rows and columns. Genotypic variability was assessed in terms of *P-values* (probability of observing more extreme data than can be observed under the hypothesis of no genotypic variation) using the Wald statistic. The best linear unbiased predicted estimates and their estimated standard errors were obtained. Data from the Alpha design from the two experiments in 2014/2015 and in 2016/2017 winter season were analyzed using an incomplete statistical method procedure. Genetic variance and heritability were estimated for all evaluated traits in the three winter seasons (2013/2014; 2014/2015 and 2015/2017) were computed by genetic module of Genstat 2019 using the method of residual maximum likelihood (REML) model and Best unbiased estimated value. Response to selection (RS) is estimated as the difference of mean phenotypic value between the offspring of the selected parents and the whole parental generation before selection (Falconer et al., 1960). Synthetic value was estimated using the below model described by Maalouf et al. (1999), assuming that all lines have the same level of outcrossing rate:

$$Sv_i = \frac{1}{k} v_i + \frac{k-1}{k} 2gca_i$$

Where Sv_i is the synthetic value of parent lines; v_i is parental value estimated the yield of parents under insect proof cages; gca_i is the general combining ability estimated by the offspring of Topcross in open field.

Overall differences between breeding methods were investigated by Principal Component Analysis (PCA) using best linear unbiased phenotype (BLUP) of the data obtained by the two experiments conducted in 2014/2015 and 2016/2017. The PCA is to determine the differences between synthetics, recurrent, pedigree lines and parents, to determine which traits explain most of the variations and determine whether the differences in breeding methods are associated with the patterns of variation of developed lines. The various statistics including coefficient correlation between eigen value and evaluated traits and genetic parameters were computed through REML of GENSTAT Release 18 statistical software (Goedhart and Thissen, 2018).

Results

1- Pedigree and recurrent selection methods

1.1- Genotypic variation

Genotypic variation relative to experimental error variation is presented in terms of the P-value indicating its statistical significance (Table 2). Significant differences among lines developed by the recurrent method were detected for DFLR, DMAT, PNPLT and GYPLT across generations (F3-F5). On the other hand, significant differences among lines developed by the pedigree method were observed for DMAT, BRPLT, PNPLT across generations (F3-F5). In both methods, SNPLT was significant in only the F3 and F4 generations. The selection of best lines was based on early flowering and maturing time, and on plant height and pods number per plants, compared to the best checks. These results indicated wide range of variation between populations for each trait (DFLR, DMAT, PNPLT and GY).

1.2- Genetic parameters

The Genetic variance and heritability were presented in Table 3 for the different studied traits in the three winter seasons. The genetic variances were higher in recurrent selection for DFLR, DMAT, HSW and GY in recurrent selection than pedigree methods while genetic variance for PNPLT and SNPLT were higher in pedigree methods than in recurrent. The estimated heritability h^2 was higher in case of recurrent selection in more than one season for DFLR, DMAT and HSW. For yield (GYPLT and GY kg/ha) and biological yield (BY), the genetic variance and heritability were higher in recurrent selection than in pedigree methods. These results indicated that better selection for seed size and phenological traits, yield and biological yield can be achieved in recurrent selection (presence of insect pollinators). however better selection for higher number pods and higher number of seeds might be achieved in pedigree methods under insect proof cages (self-fertility).

1.3- Response to selection

The response to selection (RS) in both recurrent and pedigree selection methods calculated from the means of selected parents and its offspring are presented in Table 4. RS varied from 4.4% to 39.2% for grain yield in the populations developed by the recurrent selection method in 2014/2015. The population S2012-85 demonstrated the highest response to selection among the population improved by the recurrent selection method. In the pedigree selection method, RS varied from 22.3% to 25.4% for the GY. The population S2012-018 had higher RS value than the other populations improved lines by this method.

2- Selection of best parental lines for synthetic development

Significant differences among lines were observed between parents for DMAT, PLHT, SNP and GY in open field and insect proof cages. Synthetic value was estimated using GY data in open field and the parental value under screen houses. 11 synthetics were formed with 3 to 11 parents using the ranking of parental lines by the synthetic value (Table 5).

3- Comparison of different breeding methods.

3.1- Univariate analysis*:* Average value per traits and methods, standard error and p-value comparing different breeding methods in two seasons are presented in Table 6. High significant differences were observed among breeding methods for BY, GY, HSW and BRPLT in 2014/2015 and 2016/2017 seasons. The average GY, BY and HSW were significantly higher in developed synthetics and recurrent lines than the pedigree and parental lines, which confirms the importance of cross pollination in increasing the variance between breeding methods for most studied traits. The synthetic lines flowered significantly earlier than recurrent, pedigree and parental lines in both seasons.

Highly significant differences ($p<0.001$) among lines were observed for BY, GY, DFLR, DMAT, HSW and SNP in 2014/2015 and 2016/2017 seasons, which indicate too wide genetic base in the studied populations for most of the measured traits. Mean values for all traits for best selected lines by different methods, average of parent lines and best check and standard error are presented in Table 6. Grains yield (GY) of tested parents varied from 2,060 to 3,553 kg/ha in 2015 and from 1,505 to 2,802 kg/ha in 2016/2017. GY of improved lines by pedigree methods varied from 1,284 to 3,100 kg/ha among lines developed by pedigree methods in 2014/2015 and from 2,151 to 3,607 kg/ha in 2016/2017 seasons. In both seasons, the lines (PE32, PE33, PE39, PE40) showed significantly higher yields than the average of the parents. For the lines developed by the recurrent method, the average GY varied from 1,649-3,710 in 2014/2015 and from 1,922 to 3,402 kg/ha in 2016/2017. Among the 30 developed recurrent lines, the yield of each of R1, R6, R9, R16, R22, R25, R28, R29, R45, R47 and R53 had significantly higher yield than the average of parents. GY varied from 2,017 to 3,488 kg/ha for first synthetic generation (Syn_0) in 2014/2015 and from 2,881 to 3,523 kg/ha for second synthetic generation Syn_1 in 2016/2017.

3.2- Multivariate analysis,

The two Principal component analyses (PCA) were used to determine which traits would differentiate between the different lines obtained by different breeding methods. The correlation coefficients between the two first principal component analyses and the studied traits and cumulative variance are reported for evaluation trails conducted in 2014/2015 and 2016/2017 in Table 8. In the first year (2014/2015) which includes, 74 lines (Syn_0, pedigree and recurrent lines, as well as parent lines, two principal components were found to explain 99.9% of the total variability. The PCA1 accounted for 90.3% of the variation and it was equally associated with BY and GY. A high PCA1 value corresponds to high values of grain yield and biological yield. PCA2 accounted for 9.6% and was almost exclusively associated to grain yield. Higher values of PCA2 correspond to higher GY. In the second year (2016/2017), which includes, Syn_1, F6 pedigree and recurrent lines, as well as parent lines with best check, the first two principal component analysis explained 99.8% of the total variability. The PCA1 accounted for 89.6% of the variation and it was positively associated with biological yield and grain yield, and negatively with days to flowering. A high PCA value corresponds to high grain yield and biological yields as well as a high value of number of branches per plant and short duration of flowering time. PCA2 accounted for 10.2% of total variability and was almost exclusively associated to grain yield. Higher PCA2 values correspond to higher GY (Figure 1)

Overall, multivariate analysis of the data indicated the most variation between lines developed by different breeding methods (synthetic lines, recurrent lines, pedigree lines) and parental lines was found to be related to biological yield and grain yield, number of branches and days to flowering. Grain yield was also reflected in the second PCA with little variation (Table 8)

In both seasons, the evaluated lines are clustered according to their performance. Only biplot of 2016/2017 season is presented as similar results were obtained in both seasons. The two first axis of the biplot allows location of the evaluated lines to be visualized in the space. During 2016/2017, the developed lines were plotted in a two-dimensional diagram and reported in Figure 1. The first PCA1 showed clear differences among developed lines differentiated by their breeding methods with respect to improvement made from their parents. The right hand of this axis is characterized by lines developed by the three studied breeding methods that have grain and biological yield, number of branches and short flowering lines. This axis chiefly indicates that synthetics have higher grain and biological yield and shorter flowering period than other developed lines. Most of the recurrent lines are in the right hand of the axis, but well separated from the synthetic. With exception of line R6, all other recurrent lines had lower BY and GY than the synthetic lines. Most of the pedigree lines coincide with their respective parents and fall in the left part of the vertical axis.

Discussions

Faba bean as a partial allogamous crop and entomophilous species can play a critical role in sustainable agriculture and in conserving wild pollinators in natural ecosystems. Faba bean breeders are seeking to determine which breeding strategy is more effective in order to achieve high yielding lines of faba bean varieties. This might include assessing the impact of breeding on the attractivity for a broader range of pollinator species or more targeted: breeding for higher nectar content, more diverse colors of petals or easer to access flowers might enhance the attractivity of faba bean in future. Studies comparing breeding strategies on faba bean have not been widely undertaken in recent years, as the recurrent selection method is barely applied on faba bean populations, whereas pedigree selection and synthetic cultivars are regarded as a major breeding method for this partial allogamous crop (Ibrahim et al. 2015; Maalouf et al. 2002). We therefore conducted this study to compare the efficiency of different breeding methods in improving faba bean productivity.

Wild bees are particularly important as crossing agents, they contribute to the expression of heterosis-mediated yield, yield stability and resilience of faba bean crop (Maalouf et al. 2008; Aouar-sadli et al. 2008; Palmer et al. 2009; Suso et al. 2005; Suso and Maalouf 2010; Nayak et al. 2014; Andersson et al. 2014; Bishop et al. 2016). In that sense, pollinators are natural breeders of the highest importance (Christmann and Aw-Hassan 2012). Development of open pollinated varieties by using recurrent selection methods (Rowland 1986) and by developing synthetics (Stelling et al. 1994, Maalouf et al. 1999) may ensure floral display diversity favoring insect pollination (Suso et al. 2005).

Recurrent selection has been used to improve cross-pollinated crops, especially to improve the performance of maize populations (Viana 2007). It has also been shown to be efficient in improving rice productivity (Morais Júnior et al. 2017). The first report on utilizing recurrent selection method in faba bean was described by Rowland (1987), who revealed that a recurrent selection program consists of growing superior lines in open pollinated random mating nurseries (RMN); selecting heavily podded plants from these lines; evaluating the offspring for yield; and replacing inferior lines in the RMN with selections deemed to be superior (Rowland, 1987). The selected lines showed a positive genetic gain of 1.8% per year. It might be possible that most single plants selected in open pollinated conditions were hybrid plants as hybrid faba bean plants are more autofertile than inbred plants (Drayner, 1959) and therefore it should produce more pods on a plant, which was the main selection criterion used in our breeding program, which was similar to the method described by Rowland et al. (1986). Our results indicated higher genetic variability for grain yield and for seed size in recurrent than synthetics and higher narrow sense heritability for most of studied traits. Higher yields in lines selected by recurrent selection than lines developed by pedigree method was observed, as the response to selection in recurrent lines was higher than those obtained by pedigree method. The possible explanation for this result may be the greater partitioning of additive genetic variance within populations improved by recurrent selection than those developed by pedigree selection. This led to the accumulation of desirable genes across generations, for the lines improved by recurrent selection rather than the lines improved by pedigree selection.

Synthetics cultivar may produce higher yield performance than breeding lines developed recurrent varieties as our results indicated. Classical breeding studies require a longer time to select individual clones than the development of synthetic varieties (Flajoulot et al. 2005). Our results indicate that the lines developed by the synthetic method yielded more than those developed by the recurrent selection method. The major reason for this might be due to the exploiting of heterosis and heterogeneity in faba bean synthetic varieties (Poulsen 1981, Stelling et al. 1994). There is also an impact for additive gene action to increase the yield in synthetic as there is no random mating among the selected parents but depending on the floral discovery, attraction and reward traits of the parents.

The partially allogamous nature of faba bean and its susceptibility to inbreeding depression (Drayner, 1959) and its autofertility characteristic meant that it could not easily be handled by the pedigree breeding method or any other breeding method used on self-pollinating crops (Lawes et al. 1983). Evaluation of the pedigree method, single seed descend, and mass selection have been conducted by different researchers (Ahmed et al. 2008; Hawtin 1982; Nassib et al. 1978). These compared methods, which are common methods for self-pollinated crops, revealed that the pedigree method was the most appropriate for faba bean (Ahmed et al. 2008). In addition, some authors have proposed transforming the mating system of faba beans (partial allogamy) towards autogamy and developing inbred lines that are pollinator independent, which are especially useful for organic agriculture uniformity and specific adaptability (Ghaouti et al. 2008). Selection for a high self-fertility degree might represent an important advantage for simplifying the breeding and facilitating seed production technology. Our results indicated that the number of selected lines obtained by pedigree methods was lower than those obtained by recurrent selections method, and the average yield gains in lines obtained by pedigree method were much lower than those developed by recurrent and synthetic methods.

Multivariate analysis provides a useful mechanism for pinpointing the components that determine the components' variation when considering several traits simultaneously. Principal Component Analysis (PCA) indicates that most of the variation among different lines developed by different methods is due to variation in biological and grain yield. According to our results, variation in yield among different lines developed by different breeding methods appeared to be based on the number of branches, biological yield and was negatively associated with days to flowering. In addition, there is little association with hundred seed weight, number of seeds and number pods per plants. Some authors found that grain yield is associated with number of pods per plants (Schill et al. 1998). Others, however, reported that hundred seed weight is associated with grain yield (Cubero and Martin 1981; Maalouf et al. 2002). In our study, we found that the variation in grain yield, biological yield, number of branches and flowering times explained the differences among lines developed by different breeding methods. In open pollinated varieties, such as synthetics and recurrent lines, yield might be associated with different functional floral traits such as keel petal dimension and floral display (Suso and del Rio 2015). Therefore, integrating an optimized keel dimension with sexual dimensions and floral display-based approaches could help enhance seed production, thereby improving faba bean food production and ecological services (Hajjar et al. 2008). Floral display and, to a lesser extent, floral design, were also considered as plant traits that are useful to improve yield (Suso et al. 2005). Recently, floral traits, in combination with pollinator behavior, have been proposed to be a useful approach to increase the level of cross-pollination (Suso and Maalouf 2010). These pollinator-mediated traits may play a critical role in attracting pollinators and increasing faba bean production.

Future implications

Breeding faba bean for sustainable agricultural production might target preserving sustainable pollinators to enhance biodiversity protection, and to be as resilient as possible to climate change effects

(Veloso et al. 2016). Pollinator independent faba bean cultivars such as self-pollinated cultivars, for instance, have higher climate change resilience, because they do not depend on insect pollinators and thus on favorable weather conditions allowing these insects to provide service, but do they adequately support pollinator protection? Large faba bean fields should provide nectar and pollen and thus sustain pollinators. Within human food, protein-rich faba bean might become even more essential as we have to shift to a balanced diet respecting the boundaries of our planet (Springmann et al. 2018).

Faba bean open pollinated cultivars are more adapted to drought-prone environments (Gasim and Link, 2007) and more tolerant to biotic stresses (Maalouf et al., 2008) and abiotic stresses (Bishop et al. 2016) than those developed under self-pollination as there is accumulation of additive genes. Higher seed size and higher yield can be achieved through the use of recurrent selection methods while higher number of pods and number of seeds per plants can be obtained with pedigree methods.

In addition, open pollinated cultivars can contribute to pollinator protection; the risks can be balanced by the FAP approach (Christmann and Aw-Hassan 2012) without external compensation for farmers (Christmann et al. 2017; Christmann 2019a; Christmann et al. in review). Local availability of wild bees might promote stable faba bean yields in the course of climate change, but how to get farmers' collaboration to restore agricultural lands as pollinator habitat without payment? The first FAP trials in Morocco on attracting higher pollinator diversity to faba bean fields using the FAP-approach are promising concerning productivity, net income, reduction of pest abundance and acceptance by farmers. The trials are currently under replication in four agro-ecosystems, publication is planned for 2020. However, shift to breeding more pollinator dependent faba bean either widens the tasks of breeders or requires further staff to ensure that farmers enhance capacity concerning threats and habitat requirements of wild pollinators and can create the optimal environment for these faba bean lines.

Producing faba bean with FAP approach can contribute to the protection of biodiversity in agricultural lands and to enhanced climate resilience of farming systems. Also environmental governance agreements between farmers in a region concerning crop rotation of cereals and faba bean ensuring that every 2000m there will be a faba bean field between cereal monocultures might contribute to pollinator protection. Breeders improving the attractivity of faba bean for pollinators might contribute in various aspects (color of petals, sugar content of nectar, easier access to the flower). We suggest that future research focuses more on the interplay of breeding and the environmental governance approach FAP.

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Conflict of interest

Authors confirm that there is no conflict of interest to declare.

Authors' contribution

Kifah Gharzeddin conducted the experiments on evaluation of different breeding methods from 2012 to 2016 and wrote the draft paper. Narjes Ali Jamal Dine conducted the experiment during 2016/2017, while preparing her Master at Lebanese University; Boulos Khoury reviewed the paper and supervised Kifah PhD. Lynn Abou Khater contributed to the experiment's implementation, data collection and analysis and to preparation of tables and list of references. Stefanie Christmann contributed to the pollinator related paragraphs in the introduction, future implication and integrated approach sections. Fouad Maalouf supervised the implementation of all experiments at ICARDA and made a major contribution to the paper writing and editing. All authors provided critical feedback to the paper.

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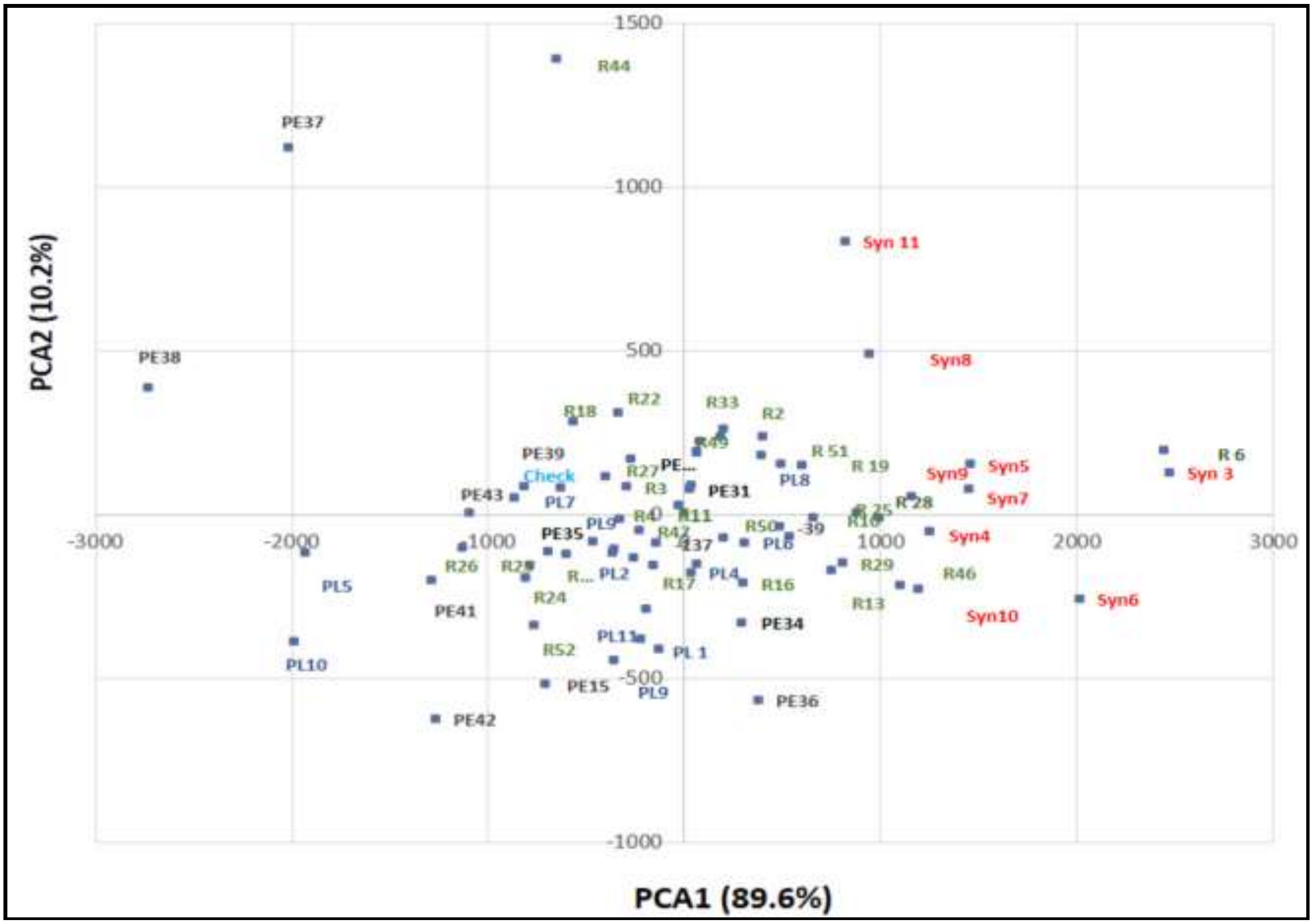

Figure 1: Biplot for Principal Component Analysis of 74 lines evaluated in 2016/2017 and showing the distribution of lines developed by synthetic (Syn), pedigree method (PE), recurrent selection (R) and parental lines (PL)

Table 1: Parental lines used in hybridization for development of F1 for further selection in different breeding methods.

Serial number	Line name	Pedigree	Origin
PL1	S2011-111	Hudieba 93 x Sel. 2010 TER.192-1	Sudan/ICARDA
PL2	S2011-112	Wadi-1 x Sel. 2010 TER 192-1.	Egypt/ICARDA
PL3	Nubaria2	ILB1550 X Radiation 2095/76	ICARDA/Egypt
PL4	Misr2	F402XBPL710	Egypt/Ecuador
PL5	Aguadolce	ILB1266	North Africa
PL6	Atuna	Population	Egypt
PL7	Sel. Br./20640-1/2010	B7/TH2009/HBP/S0/2006	ICARDA
PL8	Sel. B7/ F7/8975/05	HBP/ L.8985 / F7- 2005	ICARDA
PL9	WRB767-3-1-2-08	White flower Reina Blanca	England
PL10	S2011-107	Sel. Br./20640-1/2010 x Sel.2010 Cold 679-11	ICARDA/china
PL11	Sel.2010- Cold 679-11	ILB0-132225	China

Table 2: Spatial model analysis performed for detecting significance differences of genotypic variation in phenological and agronomical traits, expressed as P-value in development of lines through pedigree method) and recurrent selection and selection intensity (*i*)

Traits	**Recurrent selection**			**Pedigree method**		
	F3	F4	F5	F3	F4	F5
DFLR	**<0.001**	0.001	**0.0029**	0.326	0.687	**0.0029**
DMAT	**0.004**	**<0.001**	**0.0003**	**0.002**	**0.003**	**0.0003**
BRPLT	**<0.001**	**0.041**	1	**<0.001**	**<0.001**	**0.0001**
PHLT	**<0.001**	0.472	0.1469	**<0.001**	0.596	0.1469
PNPLT	0.069	**<0.001**	**0.002**	**0.025**	**0.023**	**0.0019**
SNP	0.907	0.472	1	0.421	**0.005**	1
SNPLT	0.05	0.025	0.9996	**0.013**	0.074	0.9996
HSW	0.1	0.136	0.4506	**0.004**	0. 53	0.4506
GYPLT	**0.01**	**0.015**	**0.05**	0.076	0.283	**0.006**
i	**1.37**	**1.54**	**1.54**	1.24	1.36	**1.84**

DFLR: days to flowering, BRPLT: number of branches per plant, PHLT: plant height, DMAT: days to maturity, PNPLT: pods per plant, SNP: number of seeds per pod. SNPLT: number of seeds per plant, HSW: hundred seed weight, GYPLT: single-plant yield. F3 conducted in summer 2013, F4 in winter 2013/2013 and F5 in summer 2013.

Table 3: Genetic variance ($\sigma^{2)}$) and narrow sense heritability (h^2) estimated in three winter seasons for Pedigree and recurrent selection methods

	Pedigree selection method						Recurrent selection method					
Trait	**2013/2014**		**2014/2015**		**2016/2017**		**2013/2014**		**2014/2015**		**2016/2017**	
	σ^2	h^2	σ^2	h^2	σ^2	h^2	σ^2	h^2	σ^2	h^2	σ^2	h^2
DFLR	0.00	0.00	2.993	0.27	2.893	0.62	0.361	0.38	1.129	0.15	1.33	0.34
DMAT	0.00	0.00	3.707	0.61	1.41	0.34	10.49	0.44	4.149	0.87	1.735	0.43
HSW	139	0.25	65.80	0.14	83.63	0.70	315.7	0.64	166.24	0.65	166.7	0.66
BRPLT	3.17	0.28	0.088	0.07	0	0.00	3.713	0.49	0.00	0.00	0.00	0.00
SNPLT	45	0.32	68	0.34	103.9	0.37	25.6	0.05	21.01	0.26	0.00	0.00
PNPLT	128	0.36	0.00	0.00	19.64	0.45	25.72	0.26	1.99	0.11	4.91	0.19
SNP	0.12	0.41	0.018	0.04	0.1947	0.6411	0.14	0.48	0.01	0.05	0.03	0.15
GYPLT	35.2	0.15	NA	NA	NA	NA	88.1	0.18	NA	NA	NA	NA
BY	NA	NA	181511	0.11	471687	0.37	NA	NA	395369	0.33	185792	0.30
GY	NA	NA	86225	0.17	NA	NA	NA	NA	47716	0.21	45708	0.32

DFLR: days to flowering, DMAT: days to maturity. BRPLT: number of branches per plant, , PNPLT: pods per plant, SNP: number of seeds per pod. SNPLT: number of seeds per plant, HSW: hundred seed weight, GYPLT: average single-plant yield. BY: biological yield kg/ha; GY: grain yield Kg per ha. NA: data not available

Table 4: Response to selection R in both methods estimated based on data collected in 2014/2015 between F5 and the average of parental lines

Population	Pedigree	DFLR	DMAT	BRPLT	PNPLT	SNPLT	SNP	HSW	GY
		Recurrent Selection							
S2012-001	PL1 × PL2	0.15	-1.55	-0.45	3.35	-1.66	-0.10	9.77	142.20
S2012-018	PL3 × PL8	-2.33	1.58	-0.44	1.20	3.79	0.11	2.98	339.00
S2012-019	PL4 × PL8	-1.00	-0.13	-0.87	0.89	-2.41	-0.50	35.12	834.37
S2012-079	PL5 × PL9	-2.92	-0.50	0.33	4.47	3.10	-0.75	6.27	437.83
S2012-085	PL6× PL10	-0.75	-0.17	-0.75	1.75	14.59	0.74	-5.40	1059.00
S2012-133	PL7× PL11	-2.85	-2.33	-0.42	-5.05	-3.08	0.59	19.75	177.67
		Pedigree Selection method							
S2012-001	PL1 × PL2	3.75	2.25	-1.25	6.01	7.87	-0.61	-16.05	182.00
S2012-018	PL3 × PL8	-1.13	2.75	-0.25	0.24	6.58	0.56	-4.58	438.75
S2012-019	PL4 × PL8	-1.63	0.88	-1.63	0.40	-3.28	-0.53	18.43	345.50
S2012-085	PL5 × PL9	1.50	1.38	-0.75	2.03	9.75	0.41	-12.88	475.25
S2012-133	PL6× PL10	-1.50	-1.00	-0.75	-2.61	6.44	0.82	-21.9	-601.50

DFLR: days to flowering, DMAT: days to maturity. BRPLT: number of branches per plant, , PNPLT: pods per plant, SNP: number of seeds per pod. SNPLT: number of seeds per plant, HSW: hundred seed weight; GY: grain yield Kg per ha.

Table 5: Estimation of synthetic value of faba bean progenies of Topcross design evaluated in off season 2014 and developed synthetics lines from 3 to 11 parents based on the ranking of parent lines by the estimated synthetic value (Svi)

Entry	SVi	Rank	Syn(3)	Syn(4)	Syn(5)	Syn(6)	Syn(7)	Syn(8)	Syn(9)	Syn(10)	Syn(11)
PL1	376.5	2	X	X	X	X	X	X	X	X	X
PL2	387.1	1	X	X	X	X	X	X	X	X	X
PL3	-133.8	7					X	X	X	X	X
PL4	10.6	5			X	X	X	X	X	X	X
PL5	-299.6	10								X	
PL6	-310.2	11									X
PL7	2.5	6				X	X	X	X	X	X
PL8	-240.8	9						X	X	X	X
PL9	-146.2	8							X	X	X
PL10	197.9	3	X	X	X	X	X	X	X	X	X
PL11	155.88	4		X	X	X	X	X	X	X	X

Table 6: Average, stand error and probability of significance for different traits among different methods in two seasons 2014/2015 and 2016/2017

Methods	BY	GY	DFLR	DMAT	HSW	BRPLT	PNPLT	SNP	SNPLT
	2014/2015								
Recurrent	5978	2777	107	181	117.3	3.6	15.1	2.5	36.7
Pedigree	5363	2416	108	183	98.7	3.3	15.6	2.7	40.2
Synthetics	7165	3227	107	182	103.1	4.3	15.5	2.6	40.3
Parents	6606	2723	109	182	110.5	4.0	14.0	2.6	34.0
P-value	**<0.001**	**0.001**	**0.018**	**0.093**	**0.003**	**0.043**	**0.608**	**0.615**	**0.191**
SE	**442.8**	**221.7**	**1.0**	**0.9**	**8.5**	**0.4**	**1.8**	**0.2**	**4.0**
CV%	**19.0**	**21.1**	**2.6**	**1.2**	**20.2**	**28.2**	**31.2**	**22.6**	**28.0**
	2016/2017								
Recurrent	5684	2460	105	165	93.2	5.8	16.9	3.2	52.9
Pedigree	5163	2348	105	166	87.6	4.9	17.2	3.2	52.3
Synthetics	6923	2900	104	166	105.4	6.5	18.0	3.3	57.5
Parents	5283	2199	105	167	99.9	4.9	16.1	3.3	51.4
P-value	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.485**	**0.947**	**0.136**
SE	**312.2**	**147.0**	**0.6**	**0.6**	**5.0**	**0.4**	**1.8**	**5.6**	**0.2**
CV%	**15.9**	**16.7**	**1.6**	**1.0**	**15.1**	**21.8**	**30.4**	**29.7**	**14.9**

DFLR: days to flowering, DMAT: days to maturity. BRPLT: number of branches per plant, , PNPLT: pods per plant, SNP: number of seeds per pod. SNPLT: number of seeds per plant, HSW: hundred seed weight, GYPLT: average single-plant yield. BY: biological yield kg/ha; GY: grain yield Kg per ha.

Table 7: Mean value of Biological and grain yield for all selected lines by different methods, average of parent lines and best check and standard error

Lines	Population	BY (kg/ha)		GY (kg/ha)	
		2014/2015	2016/2017	2014/2015	2016/2017
		Pedigree Lines			
PE32	S2012-018	5,276	5,663	2,758	2,679
PE33	S2012-018	5,276	5,766	3,100	2,787
PE39	S2012-018	5,139	4,852	2,832	2,311
PE40	S2012-019	6,113	5,548	3,045	2,352
		Recurrent Lines			
R-6	S2012-019	4,861	7,924	3,020	3,406
R-9	S2012-019	6,116	5,766	2,850	2,353
R-14	S2012-133	6,528	5,620	3,104	2,496
R-16	S2012-019	6,807	6,006	2,993	2,371
R-22	S2012-018	7,362	5,240	3,575	2,671
R-25	S2012-085	6,667	6,282	3,296	2,669
R-28	S2012-019	6,665	6,491	2,869	2,751
R-29	S2012-019	7,083	6,470	3,114	2,582
R-45	S2012-019	6,391	5,664	3,180	2,715
R-47	S2012-001	6,111	5,452	3,711	2,363
R-53	S2012-019	6,520	-	2,958	-
		Synthetic Lines			
	Syn3	4,862	7,971	3,007	3,349
	Syn4	6,947	6,863	3,437	2,809
	Syn5	8,057	6,997	3,144	3,067
	Syn6	7,493	7,652	3,433	2,843
	Syn7	--	7,018	--	2,993
	Syn8	6,667	6,409	3,340	3,232
	Syn9	9,167	6,740	3,465	2,881
	Syn10	7,084	6,862	3,388	2,623
	Syn11	7,221	6,184	3,488	3,523
Parent means		6,603	5,265	2,722	2,176
Best check means		7,778	4,815	2,614	2,267
Standard error		195.8	243.8	83.56	97.87

BY: Biological yield; GY: Grain Yield

Table 8: Correlation between different analyzed traits and the two major principal components with percentage variation in the 2014/2015 and 2016/2017 seasons.

	2014/2015			2016/2017	
	PCA1	PCA2		PCA1	PCA2
Biological yield (kg/ha)	**0.99**	-0.11		**0.99**	-0.11
Grain yield (kg/ha)	**0.71**	0.70		**0.68**	**0.73**
Days to flowering (DFLR)	-0.07	-0.15		**-0.39**	0.10
Days to maturity (DMAT)	0.09	-0.18		-0.04	-0.21
Hundred seed weight (HSW)	0.21	0.15		0.19	-0.02
Number of branches per plant (BRPLT)	**0.24**	0.01		**0.49**	-0.20
Number of pods per plant (PNPLT)	0.08	0.07		-0.13	0.06
Number of seeds per pod (SNP)	0.05	0.20		0.15	0.03
Number of seeds per plant (SNPLT)	0.13	0.25		-0.08	0.12
Percentage variation (%)	**90.3**	**9.6**		**89.6**	**10.2**