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Effect of Heterogeneity and Heterozygosity on Agronomic Performance of the Semi-arid Grown Faba Bean (*Vicia faba* L.)

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Abstract: In faba bean, the genotypically heterogeneous structure of its population has an important impact on yield performance and remains the major constraint a plant breeder has to contend with to improve the crop yield. The aim of this study was to evaluate the effect of heterogeneity and heterozygosity on yield performance of the semi-arid grown faba bean. Five faba bean inbred lines (in S_6 generation), F_1 and F_2 generations as well as mixtures formed by blending an equal amount of seeds from each of them to produce line mixture, F-1 mixture and F2-mixtures were used to execute the experiment. The seeds of the pure lines, lines mixture and F1-and F2-mixture were grown in two consecutive seasons (2014/2015-2015/2016) at the Experimental Farm, Faculty of Agriculture, Shambat in a randomized complete block design with three replicates. Data were collected on yield and yield components. Heterogeneity in lines mixture caused a positive and significant increase of +16% in number of pods per plant and 11% increase in both 100-seed weight and yield compared to pure lines performance, whereas heterogeneity in "pure lines+ F1-hybrid and that in pure lines mixture+ F1-hybrid mixture gave significant positive yield of 28% and 24% , respectively when compared to the performance of F2 and F2 mixture. Heterozygosity in

F1 gave significant positive yield increase of 67% when compared to lines performance, whereas heterozyosity in F1-mixture gave significant yield increase of 60% when compared to lines mixture. The same effect was shown by yield components. The results clearly indicate the great positive effect of heterozyosity compared to that of heterogeneity, which indicate the possibility of production of synthetic cultivars as alternative to the still not yet commercially produced hybrids in faba bean.

Key words: faba bean, heterogeneity, heterozygosity, semi-arid condition, synthetic cultivar, yield

INTOUDUCTION

Faba bean (*Vicia faba* L.) is one of the most important grown and consumed crop legumes in the Sudan and the Middle East because of its high protein content. Moreover, cultivation of the crop has a positive effect on the environment and soil fertility through the release of residual nitrogen fixed by symbiotic Rhizobia (Oliveira *et al*. 2016). In Europe, cropping of faba bean is increasingly encouraged to achieve self-sufficiency with respect to protein feed components that is demanded by organic livestock standards (Hancock *et al*. 2005). In Sudan, despite the great efforts that have been devoted to yield improvement in faba bean, low yield and yield instability of the released cultivars remain the major constraints in the crop cultivation (Khalifa *et al*. 2017). Breeding for yield stability in faba bean is considered as the most important factor for improvement by making yield less dependent on weather (Stelling *et al*. 1994; Knot 1996; Soon-Jae Known *et al*. 2010; Torres and Carmen 2012). In addition to the effect of heat stress, drought and pests and diseases, the mix mating system of the species (self- and cross-pollination) is considered as the most important factor affecting crop productivity (Abel and Link 2002; Gasim *et al*. 2004; Link and Ghaouti 2012). Because of this type of mating system, faba bean cultivars/accessions are genetically heterogeneous and differ extremely in their inbreeding coefficient and heterozygosity as well as in their degree of cross-fertilization (Link and Von-kittlitz 1989; Link *et al*. 1994a; Abel and Link 2002; Gasim *et al*. 2004).

In breeding, this genetic structure leads to the problem that the genotypic variance of heterotic traits is larger than the heritable variance, *i.e.*, the superiority of the heterotic traits is mostly due to their high level of heterozygosity and to a lesser extent because of superior genes (Abel and link 2002; Gasim *et al*. 2004). Therefore, mass selection of superior individuals is not promising in a partially allogamous faba bean (Link *et al*. 1994b; Gasim and Link 2004; Link and Ghaouti 2012). In other partially allogamous crops like rapeseed, sorghum and cotton, breeders make use of heterosis in commercial hybrid production.

In spite of the occurrence of high heterosis in yield and yield components in faba bean (Ebmeyer 1988; Link *et al*. 1994b; Stelling 1997; Obiadalla-Ali *et al*. 2013; Gasim and Mohamed 2018), the full exploitation of hetesosis in commercial hybrid production is not yet feasible due to instability of cytoplasmic male sterility (Duc *et al*. 1992; Link 2006; Gnanasambandam *et al*. 2012). In Europe, Link *et al*. (1994c) and Stelling *et al*. (1994) pointed to the improvement of yield and yield stability in faba bean by increasing the levels of heterozygosity and heterogeneity by breeding synthetic cultivars. The objective of the present study was to evaluate the effect of heterogeneity and heterozygosity on agronomic performance of the semi-arid grown faba bean populations.

MATERIALS AND METHODS

Five inbred lines (S_6 generation) produced from a single seed descent from the widely cultivated faba bean cultivars in Sudan (Selaim, Hudeiba, Ed-Damar, Bassabier and Shabah) were used in this study. These lines were sown in isolated cages for multiplication (by hand tripping) and crossing purposes by hand in all possible combinations (in diallel cross, excluding reciprocals) to produce F_1 seeds. Also F_2- generations were produced by hand tripping. From these entries, *i.e.*, Lines (L), F_1 and F_2, mixtures were produced by blending an equal number of seeds (25 seeds) from each of them to produce line-mixture (L-mix.), F_1-mixture (F_1-mix.) and F_2-mixture (F_{2}-mix.). Altogether, 27 entries were produced (no data for one of the F2s). The seeds of the pure stands of the lines, F1 and F2 as well as the L-mix, F1-mix and F2-mix were sown in the 3rd week of November in two consecutive seasons (2014/2015- 2015/2016) in a randomized complete block design with three replicates. The gross plot size was $6.3m^2$ consisting of 3 ridges, 3m in length and 0.7m apart. One seed was planted per hole with the spacing of 20 cm along the ridge. The experimental plots were irrigated every 10 days and hand weeded whenever required. The site of the experiments was the Demonstration Farm of the Faculty of Agriculture, University of Khartoum, Shambat (latitude 15° - 40′ N; longitude 32° - 32′ E, and altitude 380m above sea level). The soil of the site was alkaline clay (pH=8.5) and the climate is semi-arid with a mean annual rainfall of 100- 200m (rainy season, July-September) with maximum temperature of 42ºC in summer and around 21°C in the winter

Data collection

Data were recorded, in the mixtures and in their components in pure stands; on i) days to flowering, determined when 50% of the plants of each entry open the first flower, ii) plant height measured in cm at the end of the flowering period, iii) number of branches per plant, iv) number of pods per plant, number of seeds per pod, vi) 100-seed weight (g) and vii) seed yield t/ha.

To simulate the population structure in faba bean, heterogeneity and heterozygosity were created using the approach of Stelling *et al*. (1994). Accordingly, and for the effect of heterogeneity, four groups of entries differing in their inbreeding coefficients were developed and assessed; they were: i) line mixtures compared to the corresponding pure lines, *i.e.*, genotypically heterogeneous entries, but homogenous for the inbreeding coefficients of their individuals ii) F_1-mixtures compared to the corresponding pure F_1s (individuals homogenous for their inbreeding coefficient), iii) pure F_2 compared to the average of the corresponding pure F1-hybrids and lines (individuals differing slightly in their inbreeding coefficient) and, iv) F1-hybrid mixture and lines compared to the corresponding F_2 mixture (more heterogeneous entry with different inbreeding coefficients). The effect of heterozygosity was assessed with the comparisons of: i) pure F_1versus pure lines (homogenous population) , and ii) F_1 mixtures versus lines mixture (heterogeneous population).

Data analysis

The collected data from the target parameters were subjected to statistical analysis following the method described by Gomez and Gomez (1984) for the randomized complete block design (RCBD). Combined analysis of variance, for the two seasons, was carried out using SAS software (1997) version 9.0.

RESULTS AND DISCUSSION

Analysis of variance (Table 1) showed that there were significant differences among the entries for most of the traits. Heritability estimate among the lines was 0.7 for number of pods per plant, 0.74 for number of seeds per pod, 0.89 for yield, and 0.93 for 100-seed weight and 0.95 for plant height (Table 1). Heterogeneity in Line mixtures showed significant increase of 11% in both 100-seed weight (+7g) and yield (+0.36 t/ha) and an increase of 16% (+3.80) in number of pods per plant compared to the performance in pure lines (Fig.1 and Table 2). However, no significant differences were detected in other traits. When F1-hybrid mixtures were compared to pure stand of F1-hybrids, the effect of heterogeneity was not significant in all traits as the entries share the same parental lines (Fig. 2). Similar heterogeneity effects in yield increase in lines mixture versus pure lines were reported by Link *et al*. (1994c). In addition to the effect of heterogeneity, Link *et al*. (1994c) attributed such yield increased to the effect of epistatic gene action.

Heterogeneity at heterogeneous level, when F_2s were compared with the average of “F_1s+lines”, caused significant increase in all of the studied traits (Fig.3). Positive increases of +1.57 (33%) in number of branches per plant, +4.30 (15%) in number of pods per plant and +1.00 t/ha (24%) in yield were registered for the average performance of "F_1s+lines" compared to that of the F_2 (Table 2). The increase in performance of the "F_1s+lines" entry over the F_2 is ascribed to the more heterogeneous structure in the "F_1s+lines" population that may result from some variation in inbreeding coefficients of

Table 1. Mean squares from analysis of variance for yield and yield components in twenty seven faba bean entries d.f = degree of

Source of variation	df	Character						
		DFL	PH(cm)	BP	PP	SP	100SW(g)	GY t/ha
Replication	2	0.049	20.804	0.054	1.002	0.005	0.375	0.023
Entries	26	0.451ns	88.190**	3.919**	168.896**	0.018**	40.013**	2.912**
Lines	4		74.589**	0.399ns	38.392**	0.023*	93.934**	0.057**
L-mix vs lines	1		0.169ns	0.312ns	16.900**	0.003ns	79.901*	1.695**
F_1s *vs* lines	1		1842.480**	50.99**	2362.750**	0.157**	390.060**	58.952**
F_1s	9		58.296**	1.540ns	148.755**	0.002ns	8.194**	0.554*
F_1-mix *vs* F1s	1		4.945ns	0.97ns	0.198ns	0.002ns	11.462ns	0.360ns
F_1-mix *vs* L-mix	1		151.002*	0.807*	235.627*	0.014ns	6.202ns	2.294ns
F_2s	8		41.267**	0.387ns	58.869**	0.008ns	11.095*	0.560*
(F_1+Lines) *vs* F_2s	1		434.049*	42.123**	652.422**	0.081*	113.330**	6.628**
(F_1+L)-mix *vs* F_2-mix	1		75.645*	4.205*	32.00*	0.045ns	168.914**	2.007*
Error	52	1.869	7.619	0.942	0.980	0.002	1.708	0.132
h^2(lines)		-	0.95	-	0.71	0.74	0.93	0.89

freedom, DFL=days to 50%flowering; PH= plant height (cm), BP= number of branches per plant, PP= number of pods per plant, SP= number of seeds per pod, 100SW= 100-seed weight (g) and GY=grain yield (t/ha). *and **= significant difference at 0.05 and 0.01, respectively; ns= non-significant difference.

(-)= not available data; one of the F_2 entry was missing; h^2= heritability

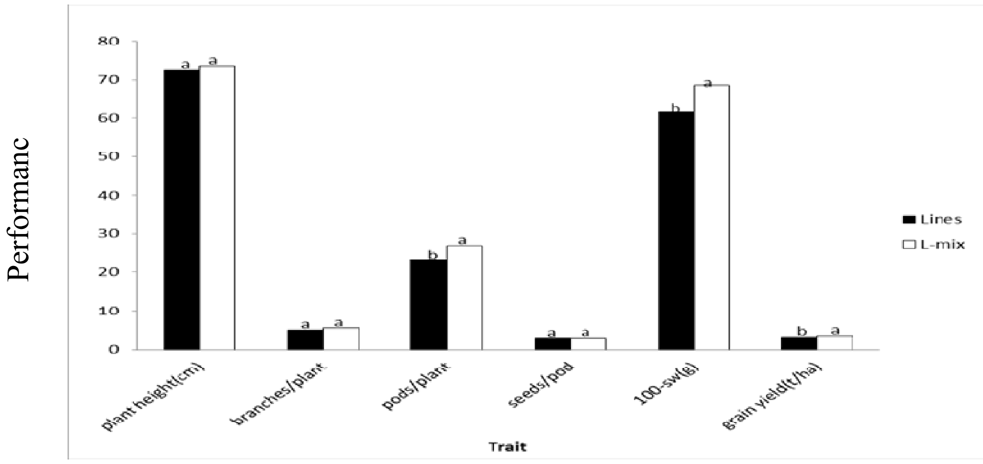

Fig.1 Mean yield and yield components in lines versus line-mixture of faba bean.

Table 2. Effect of heterogeneity on yield and yield traits in faba bean

Entries / trait		PH cm	BP	PP	SP	100-SW (g)	GY t/ha
Ls-m *vs* Ls	I	+1.00	+0.52	+3.80	+0.04	+7.00	+0.36
	II	101%	110%	116%	101%	111%	111%
F_1s-m *vs* F_1s	I	+0.70	+0.33	+1.00	+0.02	+2.03	+0.36
	II	101%	105%	103%	101%	103%	107%
(F_1s+ Ls) *vs* F_2s	I	+5.00	+1.57	+4.30	+0.10	+1.94	+1.00
	II	107%	133%	115%	103%	103%	128%
(F_1s+Ls)-m *vs*	I	+1.60	+1.00	+3.5	0.05	+3.80	+0.87
F_2s-m	II	102%	119%	112%	102%	106%	124%

Ls=lines ; Ls-m=line mixture; F_2s-m=F_2s mixture; (F1s+Ls)-m=F_1s+lines mixture; I=absolute difference in performance; II= performance of more heterogeneous entry as percent of more homogeneous one

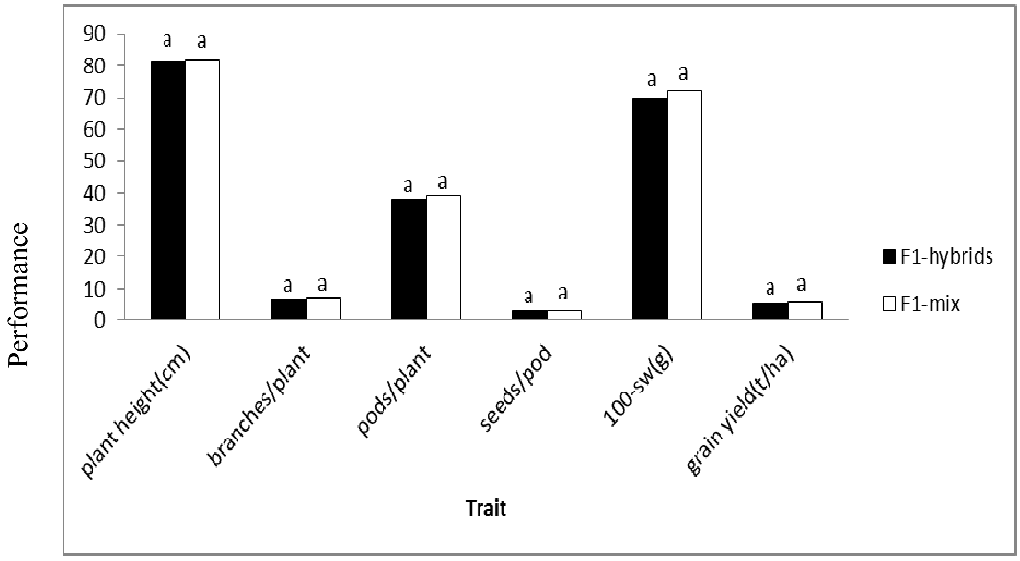

Fig. 2. Mean yield and yield components of F1-mix versus F_1s-hybrid of faba bean.

their individuals compared to the heterogeneity of the F_2 individuals, where the population is genetically heterogeneous, but homogenous for their individuals' inbreeding coefficient (Link *et al.* 1994c). Similarly, Compared to F_2-mixture, F_1s+lines-mixture (more heterogeneous level) showed an increase of +0.87 t/ha in yield (28%), +3.80g (6%) in 100-seed weight, +3.50 (12%) in number of pods per plant and +1.00 (19%) in number of branches per plant (Fig.4 and Table 2). Again, this marked significant increase in performance of "F_1+L-mixtures" compared to the performance of F_2-mixture could be ascribed to the heterogeneity in inbreeding coefficient of "F_1+L-mixtures" individual components as well as to the interaction of heterozgosity and heterogeneity (Ebmeyer 1988; Link and Ederer 1993; Stelling *et al.* 1994 and Gasim and Mohamed 2018).

For the effect of heterozygosity [comparison of homogenous populations (F1-hybrids versus lines) and heterogeneous population (FI-mixtures versus L-mixtures)], analysis of variance showed significant differences in most of the traits, except for the number of seeds per pod in the comparison of F1-mixutre versus L-mixture, which showed no significant difference (Table 1). At the homogenous level, F1-hybrids exhibited significant positive increase

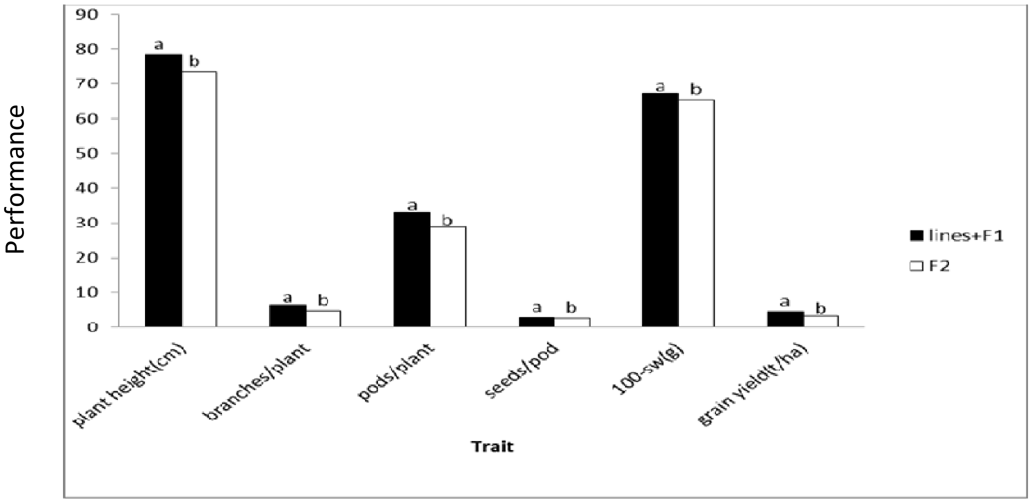

Fig.3. Means of Lines+ FI-hybrids versus F2-hybrids for yield and yield components in faba bean.

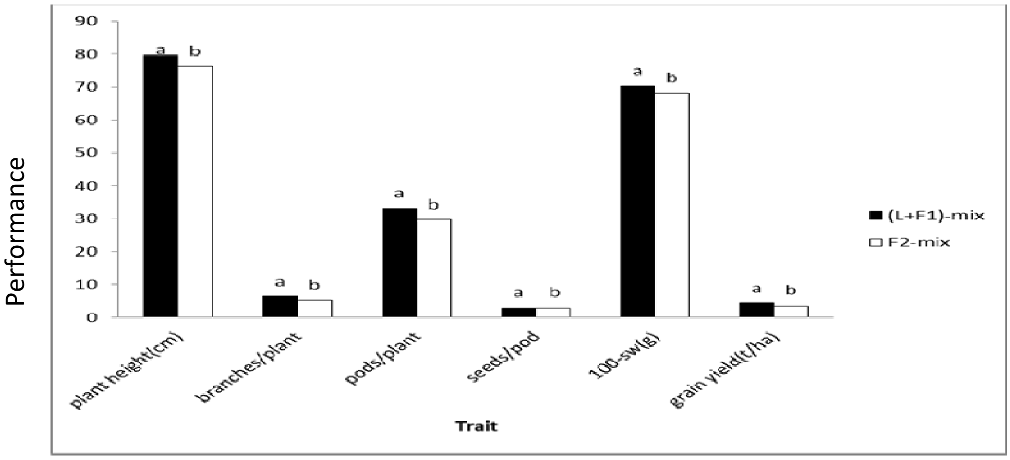

Fig.4. Means of (F1+Line)-mix versus F2-mix for yield and yield components in faba bean.

of +9.00 cm (12%) in plant height, +8.50 g (14%) in 100-seed weight, + 1.70 (35%) in number of branches per plant, +14.80 (64%) in number of pods per plant and +2.10 t/ha (67%) in yield compared to lines performance (Fig. 5 and Table 3).

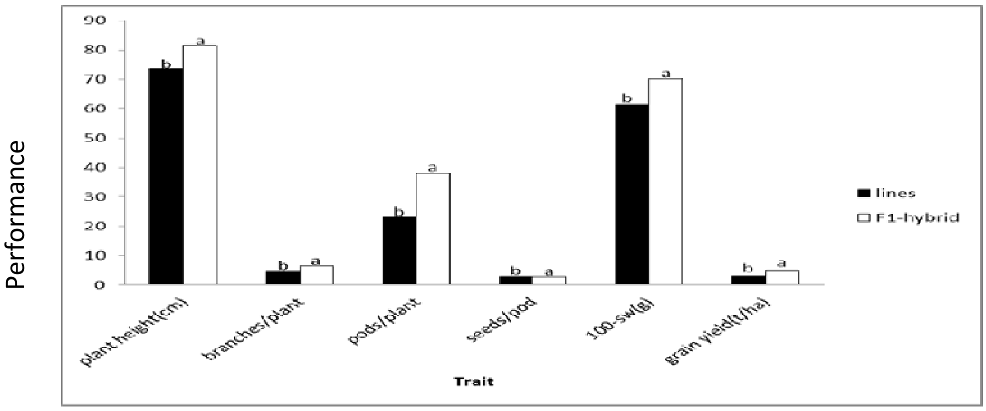

Fig. 5. Means of FI-hybrid versus Lines for yield and yield components in faba bean.

Table 3. Effect of heterozygosity on yield and yield component in faba bean

Entries/Trait		PH (cm)	BP	PP	SP	100-SW (g)	GY (t/ha)
F_1s vs Ls	I	+9.00	+1.70	+14.80	+0.10	+8.50	+2.10
	II	112%	135%	164%	102%	114%	167%
F_1s-m vs Ls-m	I	+8.70	+1.20	+12.30	+0.10	+3.6o	+2.07
	II	112%	122%	146%	103%	105%	160%

Ls=lines; Ls-m= lines mixture; F_1s-m= F_1s-mixture; I= absolute difference in mean performance; II= mean performance of heterozygous entry as percent of homozygous entry

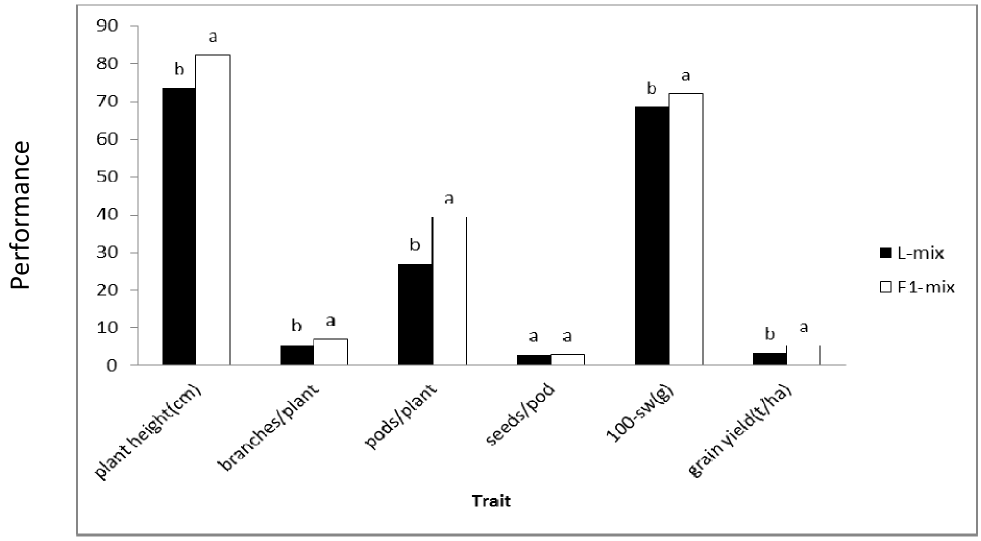

Fig. 6. Means of FI-mix versus L- mix for yield and yield components in faba bean.

At heterogeneous level, F1-mixtures surpassed the performance of line mixtures with +8.70 cm (12%) in plant height, +1.20 (22%) in number of branches per plant, +12.3 (46%) in number of pods per plant, +3.6 g (5%) in 100-seed weight and +2.07 t/ha (60%) in yield as shown in Table 3 and Fig 6. Comparing heterozygosity at homogenous population structure with that of the heterogeneous one, the heterotic performance of studied traits was slightly higher in homozygous population than in heterogeneous population as reported by link *et al*. (1994c).

Generally, comparing the effect of heterogeneity and heterozygosity on yield increase, the effect of heterozgosity was much higher than the effect of heterogeneity. However, Stelling *et al*. (1994) pointed to the minor joint effect of the interaction of heterogeneity and heterozygosity in F1-mixtures. The results indicate that yield increase in faba bean is mediated by heterosis and could be improved by increasing the level of heterozygosity (Link *et al*. 1994c; Suso and Maalouf 2010). Gasim and Mohamed (2018) reported that mid-parent heterosis at the same experimental site, was 67% for yield, 64% for number of pods per plant, 12.33% for plant height and 10.5% for 100-seed weight with the degree of cross-fertilization on average of 36%. Hence, these data together with the present ones point to a large potential advantage of hybrid cultivars in this material compared to the current use of line cultivars. Therefore, exploitation of heterosis in faba bean could improve its yield and yield components. However, as stable cytoplasmic male sterility is still to be discovered for full exploitation of heterosis in successful hybrid production and eventually yield increase (Link 2006), synthetic cultivars bred from the present material may realize about 75% of total amount of heterosis (Link and Ederer 1993; Gasim *et al*. 2004). Such cultivars could be an alternative to the still not yet bred hybrid cultivar as the prerequisites for their production are present in the current material (Gasim and Mohamed 2018). The present results follow the previous knowledge on the topic. Further investigations on the effect of environment on yield performance are needed.

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تأثير الخلط الوراثى و تباين العوامل الوراثية فى الأَداء الحقلى للفول المنزرع فى المناطق شبه الجافة

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المستخلص: الخلط الوراثى فى عشيرة نباتات الفول له دورا مهما فى اَداء المحصول ومعضلة أساسية يجب على مربة النبات وضعها فى الاعتبار لتحسين الانتاجية. هدفت هذه الدراسة لتقييم تأثيرالخلط الوراثى وتباين العوامل الوراثية فى إنتاجية الفول المنزرع فى المناطق شبة الجافة. أستخدمت خمسة خطوط نقية من الفول لإنتاج هجن الجيل الاول و الثانى إضافة لخليط تم تكوينة بخلط عدد متساوى من البذور من كل منها لإنتاج خليط الخطوط النقية و خليط هجن الجيل الاول و الثانى لتنفيذ التجربة. زرعت بذور الخطوط النقية وخليط الخطوط النقية وخليط هجن الجيل الاول والثانى بالمزرعة التجربية بكلية الزراعة، شمبات لموسمين متتالين (2015/2014 و 2016/2015) بإستخدام تصميم القطاعات الكاملة بثلاثة مكررات. جمعت بيانات الانتاجية و مكوناتها من كل المداخيل. الخلط الوراثى فى خليط الخطوط النقية تسبب فى زيادة معنوية موجبة مقدارها 16% فى صفات عدد القرون فى النبات وزيادة +11% فى كل من وزن 100- بذرة و الانتاجية طن/هكتار مقارنة بأداء الخطوط النقية، بينما الخلط الوراثى فى الخطوط النقية + هجن الجيل الاول وذاك الذى فى خليط الخطوط النقية + خليط هجن الجيل الاول أعطى زيادة معنوية وموجبة مقدارها 28% و24% عند مقارنتها باداء الجيل الثانى وخليط الجيل الثانى، على التوالى. تباين العوامل الوراثية فى هجن الجيل الاول أعطى زيادة معنوية موجبة فى الانتاجية مقدارها 67% مقارنتها بأداء الخطوط النقية، بينما أعطى الخلط الوراثى فى خليط الهجن زيادة معنوية موجبة فى الانتاجية مقدارها 60% عند مقارنها باداء خليط الخطوط النقية. أعطت مكونات الانتاجية نفس التأثير. توضح النتائج بجلاء التاثير الكبير لتباين العوامل الوارثية فى إنتاجية الفول مقارنة بتأثير الخلط الوراثى ، مما يشير لإمكانية إنتاج الاصناف المركبه كبدائل جاذبة للهجن التي لم تنتج على المستوى التجاري في الفول حتى الآن.