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Evaluation of the genetic variability of faba bean (*Vicia faba* L.) genotypes using agronomic traits and molecular markers

Adel Ahmed Mohamed Elshafei¹, Mohamed Abd Elaziz Amer², Magda Aly Mahmoud Elenany^{1*} and Ahmed Gamal Abd Elhameed Helal³

Abstract

Background: Breeding programs need to work with magnitudes of genetic variation in order to achieve the best results. In this study, we have estimated the genetic diversity and evaluated the field performance of 18 faba bean genotypes from different origins, during the 2014–2015 and 2015–2016 growing seasons.

Results: The field evaluations revealed that the faba bean genotypes showed enough variation for their agronomic thoughtful parameters. ALB was noted as being the genotype that was the tallest (126.25 cm), but the superlative genotypes were L251 and L252 for a high number of seeds per plant (72.25) and the maximum seed yield per plant (56.40 g). The estimations of the genetic diversity between all of the faba bean genotypes were done using eight sequence-related amplified polymorphism (SRAP)-combined primers. The number of different alleles amplified for each polymorphic marker, ranged from 2 to 9, was observed by SRAP genotyping, with polymorphic information contents (PIC) between 0.445 and 0.896, and discrimination power (DP) with an average of 0.125 per allele.

Conclusions: Across both years, the best grain yields were gathered from the L25, L252, and L182 genotypes, in descending order, whereas the ALB genotype had the lowest grain yield in comparison with the other genotypes. The cluster analysis of faba bean genotypes was generally based on not only the origin but also the genetic background.

Keywords: Genetic diversity, Agronomic traits, Faba bean, SRAP, PIC

Background

Faba bean (*Vicia faba* L.) is one of the most remarkable crops for its seed nutritional value, as it has a high protein content and is considered to be one of the main sources of protein in the human diet (Crepona et al. 2010). It also has value as an export crop for feed markets (Gong et al. 2011). The world cultivated area for faba bean was 2.55 million hectares, which yielded a total output of 4.3 million tons (FAOstat 2012). The average cultivated area over the last 5 years (2012–2017) was about 113,000 fed, with an average yield of 9.2 ard fed⁻¹; the total production was 119,000 ton; and the consumption was 420,000 ton. It is essential to expand the production of faba beans to meet the high local demands for consumption, and this expansion needs to

occur throughout reclaimed areas that are considered to be cultivated land (Khalafallah et al. 2008; Bakry et al. 2011). Utilizing a selection of faba beans that have variations in their morphological attributes such as flowering, number of pods, number of seeds, and seed size may be useful in the construction of selection indices for the improvement of faba bean yields (El-Hady et al. 1998).

In some faba bean landraces, their genetic diversity has been assessed using different molecular markers such as RAPD, SSR, EST-SSR, TRAP, IRAP, and AFLP (Dixkens et al. 1995; Akash et al. 2017; Kwon et al. 2010; Tomas et al. 2016; Zong et al. 2009) which used 12 TRAP primer combinations amplified. A high level of polymorphism was discovered among the accessions studied, with a predestined average pairwise similarity of 63.2%, ranging from 36.9 to 90.2% (Tomas et al. 2016).

Kwon et al. (2010) used 15 SSRs to assess the variation and structure of 35 faba bean landraces originating from

* Correspondence: menany245@hotmail.com

¹Genetics and Cytology Department, Genetic Engineering and Biotechnology Division, National Research Centre (NRC), El-Dokki, Cairo, Egypt
Full list of author information is available at the end of the article

3 geographically different areas (East Africa, North Africa, and the Near East). They found high diversity levels in the analyzed landraces of faba bean and identified that they could be utilized in upcoming breeding programs to encourage the development of new plant varieties from selective breeding. Inter-simple sequence repeat (ISSR) markers were also used to report and analyze the genetic diversity of the faba bean accessions (Terzopoulos and Bebeli 2008; Guan et al. 2011; Salazar-laureles et al. 2015; Asfaw et al. 2018). The ISSR markers were found to be effective in identifying the diversity between the bean accessions and allowing for specific contrasting accessions to be used in genetic refinement programs, to satisfy the needs of the producers (Salazar-laureles et al. 2015). The results of the ISSR data showed that the faba bean landraces were highly linked genetically via their geographical regions and their ecological behaviors (Wang et al. 2012).

The evaluation of genetic variability among faba bean landraces using agro-morphological and molecular markers revealed that sequence-related amplified polymorphism (SRAP) and amplified fragment length polymorphism (AFLP) markers are used to determine the genetic variability among genotypes (Ammar et al. 2015; Backouchi et al. 2015; Abid et al. 2015; Rebaa et al. 2017; Alemu et al. 2018). AFLP markers and combined SRAP are considered to be remarkably beneficial for the evaluation of genetic variability at the molecular level. They demonstrate high discrimination power (DP), differentiating the faba bean genotypes with high effectiveness and accuracy levels. It has been reported that 17 SSRs and 15 morphological traits could be used to evaluate the genetic variation among faba bean genotypes originating from different countries, and they were included in the International Center for Agricultural Research in the Dry Areas (ICARDA) breeding program (Ammar et al. 2015; Abid et al. 2015). Significant differences among the genotypes of the 15 morphological descriptors were identified. The SSR marker analysis indicated high genetic variability among the accessions. The existence of genetic variability was evident, from the morphological and molecular evaluations, suggesting the possibility of crossbreeding genetically diverse varieties (Alemu et al. 2018). The genetic differences determined using 23 phenotypic traits and 11 (ISSR) primers could be used in faba bean breeding programs by desegregation with other germplasm resources for faba bean improvement. Using ISSR data as a basis, the overall results indicated that the genetic relationship of faba bean genotypes was highly linked to their geographical regions of origin and their ecological behaviors (Wang et al. 2012).

This study aims to evaluate the field performance of 18 faba bean genotypes and varieties, using agronomical performance measures and SRAP molecular markers, to investigate their genetic variability.

Methods

Plant materials and field conditions

The experiments for this investigation were all conducted over two growing seasons 2014–2015 and 2015–2016, at the Bahteem farm (30° 28" N, 31° 11" E), Genetic Resources Research Department, Field Crop Research Institute, Agricultural Research Center, Giza, Egypt Agricultural Research Station. Eighteen faba bean varieties were used: 13 Egyptian landraces and 5 other varieties (Nubaria1, ALB white helium-free tannin, black helium Giza3, Sakha4, and Sakha1) as shown in Table 1.

The experimental design was a randomized complete block design (RCBD) with four replications in the two seasons (2014–2015 and 2015–2016). The seeds of the faba bean genotypes were cultivated on the 10th of November for the first season and on the 15th of November for the second season. They were then planted on five-ridge plots: 3 m in length with 60 cm distances between the ridges and 20 cm apart. Standard growing practices for the region were employed, as recommended, and plots were immediately irrigated after sowing, as recommended.

Phenotypic measurements

At maturity, ten guarded plants were used to measure the agro-morphological traits: (1) plant height was measured from the soil surface to the upper most tip of the plant (PH), (2) the number of branches per plant (NB/P), (3) the first fruit node (Efne); (4) the number of pods per plant (NP/p), (5) the number of seeds per plant (NS/p), and (6) the seed yield per plant (SY/p). Homogeneity of the variance across environments was tested following Bartlett's test (Steel and Torrie 1980). Analysis was applied according to standard analysis of variance techniques for RCBD designs using MSTATC computer software, and means were separated using Fisher's protected least significance difference (LSD) test at a ' $P \leq 0.05$ ' level of probability (Steel and Torrie 1980).

Molecular marker

DNA extraction

DNA was extracted from all of the faba bean genotypes using the Wizard Genomic DNA purification kit (Promega Corporation Biotechnology, Madison, WI, USA). Then, the extracted DNA was treated with RNase and stored in a freezer at -20°C . The quality of DNA was assessed using 0.8% agarose gel and an Epoch Multi-Volume Spectrophotometer (Biotek, Winooski, VT, USA). Before conducting the SRAP analysis, the DNA was diluted to a concentration of 25 ng/ μL .

The eight SRAP-combined primers that were used to estimate the genetic diversity among the faba bean genotypes are shown in Table 5. A polymerase chain reaction (PCR) 20 μL mixture consisted of 20 to 50 ng of genomic DNA, 1 \times PCR buffer, 1.5 mM MgCl_2 , 0.1 mM of each

Table 1 Origins of the faba bean genotypes

Genotype name	Pedigree	Origin	Remarks
L-182	Unknown	Qena—18 km north Arment	Landraces
L-217	Unknown	Qena—30 km south Qena	Landraces
L-221	Unknown	Sohag—8 km north Sidfa	Landraces
L-224	Unknown	Assiut—Dairut	Landraces
V-239	Unknown	Sohag—El Bolyoul	Landraces
L-245	Unknown	Minya—12 km west El Minya	Landraces
V-248	Unknown	Qena—2 km northwest west of Naga Hammadey	Landraces
L-251	Unknown	Assiut—Bani Rafe	Landraces
L-252	Unknown	Minya—Beni Mazar	Landraces
Sakha4	Sakha1 × Giza3	FCRI	Variety
ALB	Unknown	Individual plant selection in Alegria	Variety
Giza3	Giza X New Accession 29	FCRI	Variety
Nubaria1	Individual plant selection from Rena Blanka	FCRI	Variety
Sakha1	716/724/83 X 620/ 283/85	FCRI	Variety
L-253	Unknown	Ismaelia—15 km west Zagazig	Landraces
L-258	Unknown	Zagazig—3 km west Zagazig	Landraces
V-259	Unknown	Elsharkya—6 km west Fagus	Variety
V-180	Unknown	Dakhliya—5 km west mitgammer	Variety

dNTP, 0.5 μ M each of the forward and reverse primers, and 1 U Taq polymerase in a volume of 0.025 μ L. The PCR program for the SRAP analyses included an amplification, which was carried out in a TC-5000 thermal cycler (Bibby Scientific—UK) as follows: initial denaturation at 94 °C for 5 min followed by five cycles of denaturation at 94 °C for 1 min, annealing at 35 °C for 1 min, and elongation at 72 °C for 1 min. For the remaining 30 cycles, the annealing temperature was increased to 50 °C for 1 min, with a final extension step at 72 °C for 7 min. The amplified PCR products were applied to 2% (*m/v*) agarose gel, containing 0.1 μ g cm^{-3} ethidium bromide in TBE buffer. Gels were analyzed by UVI Geltec version 12.4, 1999–2005 (USA). The SRAP data were scored on the bases of presence (1) or absence (0) of a given marker, after excluding irreproducible bands.

Molecular marker data and genetic variability

A similarity matrix (SM) was estimated according to Nei and Li (1979), using the molecular marker data as follows:

$$SM = 2N_{ij} / (N_i + N_j) \quad (1)$$

where N_{ij} is the number of bands present in both the i th and j th genotypes, N_i is the number of bands present in i th genotype, and N_j is the number of bands present in the j th genotype.

The similarity matrix was then subjected to the unweighted pair group method with the arithmetic average

(UPGMA) clustering algorithm. Principal coordinate analysis (PCoA) was used as an alternative to hierarchical clustering as the similarity matrix was used to obtain coordinates. The coordinates were then used to create scatter plots that represent the relationships among the genotypes. Both UPGMA and PCoA were conducted using PAST software version 1.62 (Hammer et al. 2001). Furthermore, to evaluate the reliability of the generated dendrograms, 1000 simulations were performed using the PAUP software version 4.0 b10 (Swofford 2001). The polymorphic information content (PIC) was calculated according to Smith et al. (2000), as follows:

$$PIC = 1 - \sum f_i^2 \quad (2)$$

where f_i is the frequency of the i th allele in the set of genotypes. The discrimination power was calculated by dividing the number of polymorphic alleles amplified for each primer by the total number of polymorphic alleles obtained (Khierallah et al. 2011).

Results

Agronomic traits

The analysis of variance revealed that genotypes (G), seasons (S), and their interactions (GXS) were all highly significant for all of the studied traits (Table 2). The average combined values of the agronomic characteristics for the 18 faba bean genotypes in the 2014–2015 and 2015–2016 seasons are indicated in Table 3. The results show a wide range of differences, as evidenced by

Table 2 Mean squared of the faba bean characters combined across two growing seasons

Source of variation	Degree of freedom	Plant height (cm)	No. of branches/plant	First fruit node	No. of pods/plant	No. of seeds/plant	Seed yield/plant (g)
Seasons (S)	1	19.51 ^{ns}	7.562 ^{**}	0.11 ^{ns}	64.000 ^{ns}	1230.840 [*]	429.180 ^{ns}
R	3	139.35 ^{ns}	1.229 [*]	1.11 ^{ns}	20.907 ^{ns}	214.599 ^{ns}	294.625 ^{ns}
Genotype (G)	17	901.35 ^{**}	2.742 ^{**}	5.371 ^{**}	286.330 ^{**}	1522.092 ^{**}	1091.431 ^{**}
GXS	17	653.89 [*]	2.504 ^{**}	8.479 ^{**}	92.838 ^{**}	698.487 ^{**}	511.050 ^{**}
Error	102	86.01	0.449	0.768	28.417	238.898	198.699

ns non-significant

**P* ≤ 0.05

***P* ≤ 0.01

Table 3 Mean performance of the 18 faba bean genotypes as a combination of the two seasons

Genotypes	Height of plant	Number of branches	First fruit node	Number of pods per plant	Number of seeds per plant	Seed yield per plant
L-182	104.50	3.500	4.875	12.875	37.125	41.088
L-217	95.62	3.125	6.125	6.625	16.000	13.488
L-221	110.62	3.375	3.875	12.125	31.500	22.175
L-224	110.75	4.000	4.125	12.250	32.125	29.075
V-239	118.75	3.620	4.375	12.750	37.125	31.850
L-245	109.37	3.000	4.375	15.875	35.750	30.163
V-248	110.62	3.375	3.875	9.625	27.750	31.713
L-251	102.00	4.250	3.375	33.000	72.250	56.400
L-252	113.75	3.500	4.125	22.125	57.625	46.888
Sakha4	107.50	3.620	3.250	11.500	33.250	27.713
ALB	126.25	3.375	6.000	10.000	16.500	5.725
Giza3	84.370	4.870	3.500	13.625	35.750	32.338
Nubaria1	109.50	4.000	4.000	11.125	35.750	36.950
Sakha1	90.620	2.500	3.250	17.250	39.250	20.175
L-253	109.37	2.625	3.750	11.500	29.000	21.925
L-258	124.37	2.875	4.750	13.125	29.500	28.975
V-259	115.62	3.005	3.750	14.000	33.750	29.588
V-180	113.25	4.000	4.125	21.125	57.375	37.925
L S D 0.05	9.197	0.665	0.869	5.287	15.329	13.98

the characteristic ranges that include plant height (PH) (84.370–126.250 cm), number of branches per plant (NB/P) (2.500–4.870), number of pods per plant (NP/p) (6.620–33.000), number of seeds per plant (NS/p) (16.000–72.250), and seed yield per plant (SY/p) (5.720–56.400 g). This signifies the presence of a wide range of genetic variability among the evaluated characteristics and highlights the genetic refinement that could be possible with the use of such a genetic pool for breeding.

The maximum plant height (PH) was recorded in the ALB variety, with a height of 126.250 cm, over both seasons, whereas the shortest plant height was recorded with the Giza3 genotype, with a height of 84.370 cm, over both seasons (Table 3). Consequently, this genotype is considered as dwarf varieties. The maximum number of branches per plant (NB/P) was recorded from Giza3 (4.870) and L251 (4.250), in the combined two seasons, followed by L224 and Nubaria1. These genotypes outperformed the average number of branches per plant (3.510) (Table 3), whereas the minimum number of branches per plant were recorded in Sakha1 (2.500), L253 (2.625), and L258 (2.875), and these genotypes had fewer branches than the general average (Table 3). The highest values of the first fruit nodes (Ffne) were recorded by genotypes L217 (6.125) and ALB (6.000), and the average value was 4.200, whereas Sakha4 (3.250) and L251 (3.375) were the smallest in the combined two seasons (Table 3).

As for the number of pods per plant (NP/p), the maximum number of pods per plant was recorded from L251 (33.000), followed by L252 (22.125), in the combined two seasons (Table 3), whereas the lowest number of pods per plant was recorded in L217 (6.625), followed by V248 (9.625) and ALB (10.000). For the number of seeds per plant (NS/p), the highest numbers were recorded from L251 (72.250) and L252 (57.625), in the combined two seasons, followed by L180 (Table 3), whereas the lowest numbers were recorded from L217 (16.000) and ALB (16.500). The largest seed weights per plant (SY/p) were recorded for the L251 (56.40 g) and L252 (46.89 g) genotypes, across both seasons, whereas, the lowest average seed weights per plant were recorded with ALB (5.725) and L217 (13.49) (Tables 3).

Correlation coefficients among the studied traits

The correlation coefficient among the six agronomic traits (PH, NB/P, Ffne, NP/p, NS/p, and SY/p) was calculated (Table 4). The correlation values of PH were significant with Ffne and NS/p but not significant with NB/P, NP/p, and SY/p. The correlation values for the number of branches (NB/P) were positively and highly significantly correlated with NP/p, NS/p, and SY/p, but were negatively but not significantly correlated with Ffne. The correlation values between the first fruit node (Ffne) and NP/p were significantly and negatively correlated, whereas they were not significantly correlated with

Table 4 Correlation coefficients of the calculated agronomic traits of 18 faba bean genotypes grown over two seasons

	PH	NB/P	Ffne	NP/p	NS/p	SY/p
PH	1.00000					
NB/P	0.09836 ^{ns}	1.00000				
Ffne	0.19204*	-0.12402 ^{ns}	1.00000			
NP/p	0.04510 ^{ns}	0.42190**	-0.18489*	1.00000		
NS/p	0.06908*	0.48013**	-0.16028 ^{ns}	0.93811**	1.00000	
SY/p	0.09540 ^{ns}	0.50103**	-0.11129 ^{ns}	0.78458**	0.87269**	1.00000

** and *** refers to significant and highly significant values, where $P \leq 0.05$ and $P \leq 0.01$, respectively

^{ns} non-significant, PH plant height, NB/P no. of branches/plant, Ffne first fruit node, NP/p no. of pods/plant, NS/p no. of seeds/plant, SY/p seed yield/plant

NS/p and SY/p. The correlation values for the number of pods per plant were highly significant and positively correlated with NS/p and SY/p, and the number of seeds per plant was significantly and highly positively correlated with SY/p.

Molecular markers analysis

Genetic diversity based on SRAP markers

Of the 11 combined SRAP primers used, 8 produced bands for generating genetic variety between the evaluated faba bean landraces. The combination SRAP primers produced a total of 47 amplified bands, with an average of 5.875 bands per primer, and had 98.25% polymorphism (Table 5 and Fig. 3). A total of 5.75 polymorphic bands with an average number of alleles for each primer pair combination across the 18 faba bean genotypes were generated (Table 5). SRAP1 combined with ME1/em4 produced the lowest number of alleles and total bands, 2 and 3, respectively, whereas, SRAP7, combined with the ME5/em2, produced the highest numbers, 9 and 73, respectively (Table 5). The PIC values measured from all of the SRAP combination primers were high and ranged from 0.445 to 0.896, with

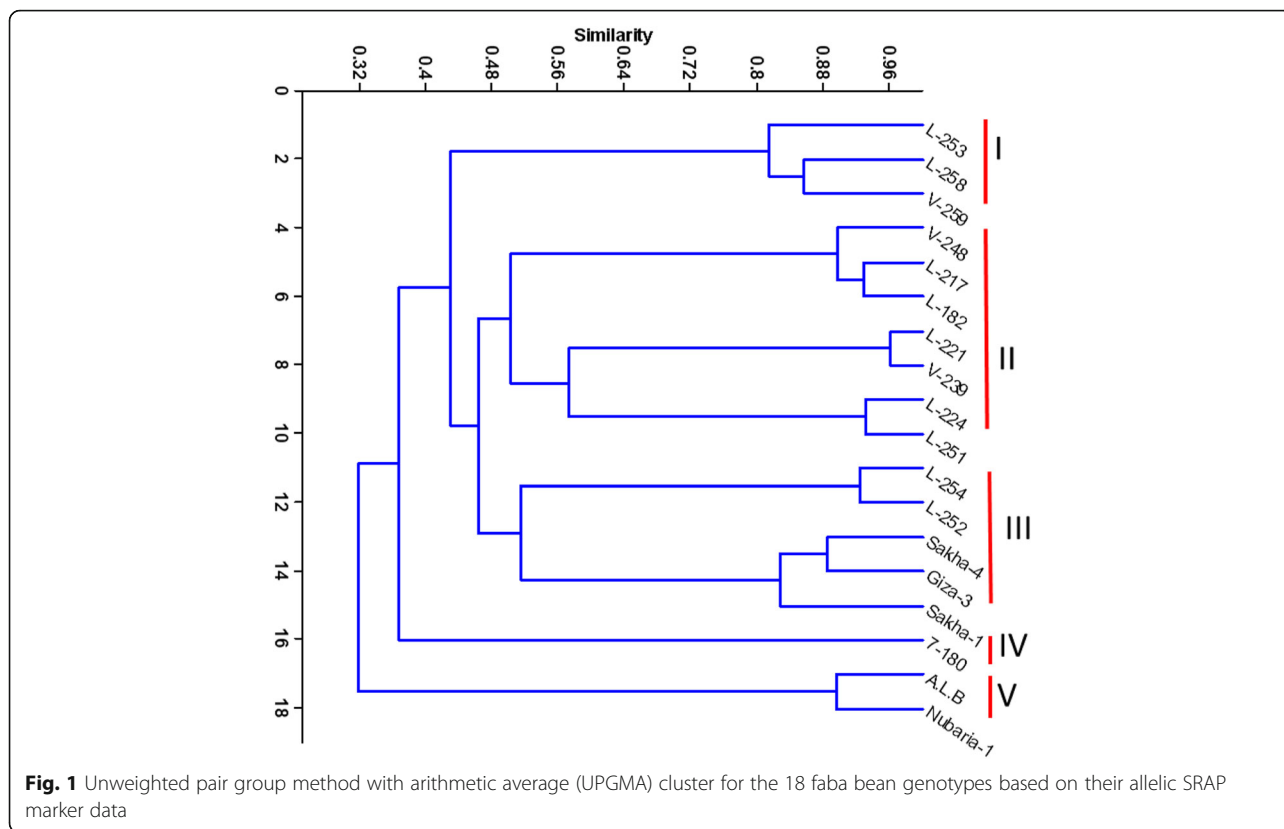
an average 0.764 per primer. The PIC values were positively correlated ($r = 0.896$), with the number of amplified alleles per primer (Table 5). The primer's DP ranged from 0.0435 for the SRAP1 combination ME1/em4 to 0.195 for the ME5/em2 SRAP7 combination, with an average of 0.125 for all of the SRAP combinations (Table 5).

A genetic similarity matrix of all of the genotypes was developed from the amplified fragments identified from the eight SRAP markers, using Jaccard's coefficients. Genetic similarity among the faba bean genotypes ranged from 0.24 to 0.96 (Fig. 1). The highest similarity values were between the genotype pairs L221 and V239 (0.96) (Fig. 1). The UPGMA dendrogram showing the genetic relationships using the SRAP markers tested among the 18 genotypes is presented in Fig. 1. At the lowest range of similarity, 0.24 among the genotype pairs ALB, Giza3, and Sakha1, the tested genotypes were divided into five main clusters. The first cluster was supported by a bootstrap value of 99% and contained three genotypes (L253, L258, and V259) that originated from the Ismaelia (L253) and Elsharkya (L258 and V259) genotypes. The second cluster, supported by a bootstrap value of 19%,

Table 5 Summary of the molecular data of the SRAP marker combinations used for the analysis of 18 faba bean genotypes

Primer name	Sequence		a	b	c	PIC	DP
	Forward	Reverse					
SRAP1(ME1/em4)	tgagtccaaccggtag	gactgctgacgaattcga	2	3	100	0.445	0.0435
SRAP2(ME2/em5)	tgagtccaaccggtcc	gactgctgacgaattcga	7	70	86	0.857	0.130
SRAP3(ME3/em1)	tgagtccaaccggtca	gactgctgacgaattctg	6	55	100	0.818	0.130
SRAP4(ME3/em3)	tgagtccaaccggtca	gactgctgacgaattaat	7	76	100	0.896	0.152
SRAP5(ME3/em8)	tgagtccaaccggtca	gactgctgacgaattggt	5	43	100	0.703	0.108
SRAP6(ME6/em1)	tgagtccaaccggacc	gactgctgacgaattctg	3	50	100	0.663	0.065
SRAP7(ME5/em2)	tgagtccaaccggtgc	gactgctgacgaattgtc	9	73	100	0.874	0.195
SRAP8(ME5/em4)	tgagtccaaccggtgc	gactgctgacgaattcga	8	75	100	0.859	0.174
Total	-	-	47	445	-	-	-
Maximum	-	-	9	76	100	0.896 to	0.195
Minimum	-	-	2	3	86	0.445	0.0435
Average	-	-	5.875	5.75	98.25	0.764	0.1247

a number of amplifications, b total polymorphic fragments (bands), c polymorphism rate (%), PIC polymorphic information contents, DP discrimination power



contained seven faba bean landraces and consisted of three subgroups. The first subgroup supported by a bootstrap value of 100% included genotypes V248, L217, and L182 that originated from the Qena genotype. The second subgroup supported by a bootstrap value of 100% included the L221 and V239 genotypes that originated from the Sohag genotype. The third subgroup supported by a bootstrap value of 99% contained two landraces L224 and L251 that originated from the Assiut genotype. The third cluster supported by a bootstrap value of 42% contained five faba bean genotypes that consisted of two subgroups. The first subgroup supported by a bootstrap value of 100% included genotypes L245 and L252 that originated from the Minya genotype. The second subgroup supported by a bootstrap value of 98% included Sakha4, Giza3, and Sakha1 genotypes that were subscribed in part of the pedigree (Table 1) and black hilum. The fourth cluster contained one genotype (V180) that originated from the Dakhliya genotype. The fifth cluster contained two genotypes (ALB and Nubaria1) that were white hilum.

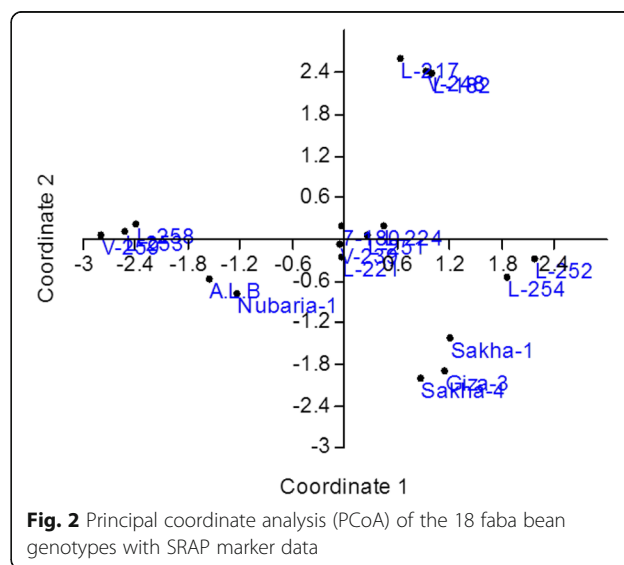
The dendrogram was confirmed by PCoA (Fig. 2). The first three PCoA for 63.8% of the total variation in accessions in the PCoA scatter plot, indicated by ellipses and numbered with A, B, C, D, and E, forms a super closed group in the dendrogram (Fig. 2). Accessions clustered in

the ellipses A, B, C, D, and E were basically from group I, II, III, IV, and V of the dendrogram correspondingly.

Discussions

Agronomical traits

In this study, the range of differences in the agronomic characteristics for plant height (84.37–126.25 cm), number of branches per plant (2.5–4.87), number of pods



per plant (6.62–33.0), number of seeds per plant (16.0–72.25), and seed yield per plant (5.72–56.40 g) was shown. This conclusion agrees with that of Musallam et al. (2004), who reported a significant variance between environments (water availability) and genotypes in the generalization of the agro-morphological characteristics and genotype X in the environmental interactions, for days to flowering and seed yield traits. Toker (2004) reported significant differences between the faba bean genotypes and all characteristics. Ammar et al. (2015) reported that all of the studied traits among the faba bean genotypes were significant, excluding that for the year the number of pods per plant and the $G \times E$ interaction. Fifteen agro-morphological traits that were examined by Akash et al. (2017) were reported to show significant variability among the faba bean accessions for pod width, pod weight, days to flowering, and grain yield. Singh et al. (2017) found that the mean squares due to the genotype were significant for all of the traits. There were highly significant differences recorded for ten yield parameters, except for the grain length and 100 seed weight (Abdullah 2018). The combined analysis of variance across the three locations evidenced highly significant variations among the 32 varieties for many of the traits (Asfaw et al. 2018).

These results were in line with the work of Tafere et al. (2012), who reported that the Gebelcho and Moti genotypes had a small number of pods per plant. However, the degaga genotype had a higher number of pods per plant. Girma and Haila (2014) found that the Shallo variety was contradicting with these results, which were lower in the number of pods per plant, whereas, the results from the Degaga variety showed the maximum number of pods per plant. Mitiku and Wolde (2015) also found that there was a variation between the faba bean varieties for pod number and plant. The results were in line with the work of Girma and Haila (2014), who found that the Shallo genotype contradicted with the results and had a lower number of seeds per plant, whereas the results of the Degaga genotype showed the maximum number of seeds per plant. Mitiku and Wolde (2015) also reported that there was a

difference between the faba bean genotypes for pod and seed numbers per plant.

The largest seed weights per plant were recorded for the L251 (56.40 g) and L252 (46.89 g) genotypes, across both seasons, whereas, the lowest average seed weights per plant were recorded with ALB (5.725) and L212 (13.49). These results are in accordance with those obtained by Tafere et al. (2012), who found that low grain yields were harvested from the Moti genotype and in the current investigation; Moti was found to be a low yielding genotype. Tamene et al. (2015) found that the Moti genotype had higher grain yields, when compared with the Degaga, Tumsa, and Gebelcho genotypes. ICARDA (2010) reported that the Wolki genotype could increase grain yields, when compared to a local check by up to 70–100%. These findings contradict the results of Girma and Haila 2014, who reported that the Shallo variety had a lower grain yield, whereas, the Degaga variety had a higher grain yield. This difference may be due to the environmental variants and the genotypes adapting to dissimilar ecological conditions. These results agree with those obtained by Dora et al. (2017) and Abdalla et al. (2015). Positive and negative correlations occur through diverse morphological and agronomic traits (Singh et al. 2017).

SRAP analysis

In this investigation, it was found that the 8 SRAP primers yielded a total of 47 amplified bands with an average of 5.875 bands per primer, and they had 97.9% polymorphisms (Fig. 3). The average number of alleles per locus (5.87) was higher compared to SSR (1.6) and SSAP markers (1.68) (Ouji et al. 2012) and the lowest compared to RAPD (31.0) and ISSR (10.9) (Backouchi et al. 2015; Asfaw et al. 2018); the results were supported by Abid et al. (2015) who showed the average number of alleles was 5.94 and ranged from 2 to 10. The percentage of polymorphic alleles was 97.9% and was higher compared to TRAP 55.2%, RAPD 75.96%, and ISSR 85.5% (Kwon et al. 2010; Elshafei et al. 2011; Backouchi et al. 2015; Asfaw et al. 2018.); they reported that using 19 SRAP primers combined with six Yucca genotypes, a

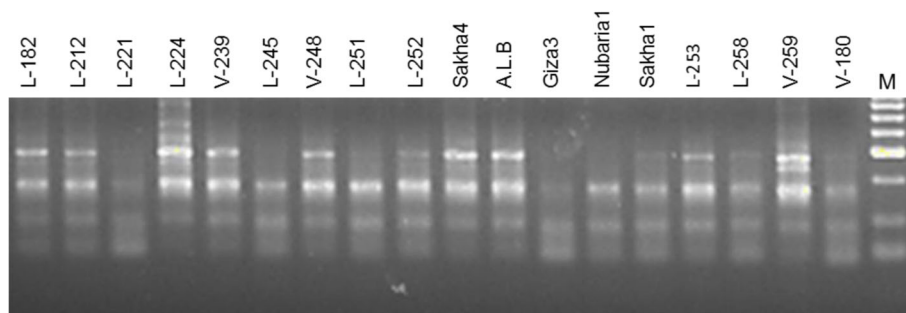


Fig. 3 Amplified products of SRAP marker using primer 3 for analyzing 18 faba bean genotypes

total of 65 alleles amplified, 72.3% were polymorphic alleles with 7.2 alleles per primer. Also, 9 TRAP primers were regenerated bands per primer ranging from 2 to 19 (Elshafei et al. 2011).

Tufan and Erdoğan (2017) found that the PIC value per primer varied from 0.15 to 0.64, with an average of 0.37 with the SSR markers. The PIC values varied from 0.38 to 0.84 with an average of 0.69, reported by Abid et al. (2015). Oliveira et al. (2016) found that the PIC value ranged from 0.071 to 0.662 with an average of 0.333. A high level of variability was found, similar to those previously recorded by Ammar et al. (2015), and primers obtained DP from 5% with primer Me2/em4 to 29% with Me2/me6, with a main 17% (Ammar et al. 2015). The polymorphism percent when the SRAP markers were used was 98.25% and was relatively larger than that of other plant species with 90% in alfalfa (Vandemark et al. 2006). The greater degree of polymorphism proved the genetic diversity among the 18 genotypes and makes the SRAP marker a perfect system for studying genetic variability among closely related genotypes. The number of amplified loci, for the 330 TRAP combinations of the primers with *castor bean* genotypes, varied from 2 to 15 with an average of 1.22 loci per combination (Simões et al. 2017).

These results suggested that cluster analysis could be used to isolate genotypes according to their geographical origin and pedigree genotypes. Kwon et al. (2010) described five major origin and pedigree groups, and Akash et al. (2017), using EST-SSR data, divided into five clusters. Tomas et al. (2016) reported cluster analysis data separated into four groups. Tufan and Tomas reported that the faba bean genotypes were divided into two major clusters, according to their origin and pedigree. Abid et al. (2015) showed that cluster analysis of genetic distances divided into two major groups and five subgroups, and recently, Asfaw et al. (2018) showed that cluster analysis separated into three clusters, according to pedigree.

The PCoA and cluster analysis distinguished these cultivars into different groups (Gong et al. 2011). The first principal coordinate accounted for 18.4% of the total variation, clearly separating the wild genotypes from the cultivated ones, and the second principal coordinate accounted for 10.4% of the total variation and consisted of all cultivated genotypes (Nandha and Singh 2014). PCoA based on ISSR data clearly differentiated Ethiopian faba bean varieties into three distinct groups. The first three groups generally divided faba beans into varieties on the basis of their pedigree (Asfaw et al. 2018).

Conclusion

Eighteen genetically different faba bean genotypes were evaluated for their yields and yield components during

the two successive growing seasons in 2014–2015 and 2015–2016. There was a high diversity among the genotypes in most parameters, whereas some genotypes were doing well at both locations. The results indicated the variations between the genotypes for most of the parameters in the combined through the two seasons. In the combined seasons, the highest grain yields were found in the L251, L252, and L182 genotypes, successively, whereas the ALB genotype had the lowest grain yields, compared to the other genotypes. The UPGMA-based dendrogram of the faba bean genotypes was generally based on their genetic background and place of origin.

Abbreviations

AFLP: Amplified fragment length polymorphism; EST-SSR: Expressed sequence tag SSR; F₁: First fruit node; IRAP: Inter-retrotransposon-amplified polymorphism; LSD: Least significance difference; NB/P: No. of branches/plant; NP/p: No. of pods/plant; NS/p: No. of seeds/plant; PCoA: Principal coordinate analysis; PCR: Polymerase chain reaction; PH: Plant height; RAPD: Random amplified polymorphic DNA; SM: Similarity matrix; SRAP: Sequence-related amplified polymorphism; SSR: Simple sequence repeat; SY/p: Seed yield/plant; TRAP: Target region amplification polymorphism; UPGMA: Unweighted pair group method with arithmetic average

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Authors' contributions

AAE and MAME performed the analysis with the molecular markers. AAH and AMA performed the field experiments. All authors read and approved the final manuscript.

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Availability of data and materials

The authors declare that the experimental data and material are available.

Ethics approval and consent to participate

The authors declare that the work is ethically approved, and consent to participate was obtained.

Consent for publication

The authors declare that the work has been consented for publication.

Competing interests

The authors declare that they have no competing interests.

Author details

¹Genetics and Cytology Department, Genetic Engineering and Biotechnology Division, National Research Centre (NRC), El-Dokki, Cairo, Egypt. ²Genetic Resources Research Department, Field Crops Research Institute, Agriculture Research Center, Giza, Egypt. ³Food Legume Crops Research Department, Field Crops Research Institute, Agriculture Research Center, Giza, Egypt.

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