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## PHYLOGENETIC ANALYSIS OF EGYPTIAN FABA BEAN CULTIVARS (*Vicia faba*) AS REVEALED BY SRAP AND SSR MARKERS

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**Key words:** Faba Bean, SSR, SRAP, and genetic diversity.

Faba bean is one of the most leading crops worldwide that originated from Asia and then was exported to Europe and Africa (Mulualem *et al*., 2012; Asfaw *et al*., 2018 and Abdalla *et al*., 2020). The expansion in Faba bean agriculture is indispensable globally not only for feeding of each human and animal but also for increasing soil fertility *via* atmospheric nitrogen fixation (Maalouf *et al*., 2011; Wang *et al*., 2012 and Salazar-Laureles *et al*., 2015).The total cultivated area of Faba bean is 2.55 million hectares with yearly production of 4.3 million tons, whereas the productivity in Egypt is about 3 tons/ha (Rebaa *et al*., 2017 and EL-Shaer and Helal, 2020). The terrible increasing in the world population must be faced *via* enhancing each the cultivated area and productivity of important legumes such as Faba bean.

It's well known that the success of any breeding program is limited to abundance of genetic variations to choose the best parents for the crossing (Cieplak *et al*., 2021). DNA markers provide plant breeders with wide range of variations to

select the favorite plant traits and therefore enhancement of their productivity (Randhawa *et al*., 2013 and Zhang *et al*., 2015). Sequence-related amplified polymorphism (SRAP) is a co-dominant marker which amplify the open reading frames (ORFs) and is distinguished with high repetition and simplicity than RAPD and AFLP (Li and Quiros, 2001; Elshafei *et al*., 2019 and Essa *et al*., 2023). Furthermore, SSRs are groups of short sequence repeats that highly polymorphic, and able to discriminate among the closed genotypes (Tahir *et al*., 2019; El-shaer and Helal, 2020 and Khalifa *et al*., 2021). It was proved that coupling of different markers is more efficient for exploiting of genetic divergence among species (Cieplak *et al*., 2021).

The Egyptian Faba bean cultivars (*Vicia faba*) (Giza 716, Giza 843, Sakha 4, Mariot 2 and Nubaria 5) are very indispensable for Egypt, distinguished with early maturing and resistance to foliar diseases. The current investigation aimed to exploit the genetic variations among five Egyptian Faba bean cultivars based on SRAP and SSR markers.

## MATERIAL AND METHODS

### Faba bean cultivars

The Egyptian Faba bean cultivars (Giza 716, Giza 843, Sakha 4, Mariot 2 and Nubaia5) were kindly provided by Prof Dr Osama Ali, Department of Agronomy, Faculty of Agriculture, Menoufia University, Egypt.

### DNA isolation

The genomic DNA of Faba bean cultivars was isolated following the instructions of DNeasy plant Mini Kit (QIAGEN).

### SRAP markers

A group of six SRAP primers were employed to detect the polymorphism among Faba bean cultivars as shown in Table (1). PCR reactions were done in 20 µl final reaction volume that contain 10 µl Master Mix, 4 ul of each forward primer (2 µl) and reverse primer (2 µl), 2 µl template DNA (10 ng) and then completed with 4 µl $dH_2O$.

PCR program was; denaturation for 5 min at 94ºC, 40 cycles (each cycle include denaturation at 94ºC for 1 m, an annealing at 40ºC for 50 sec, and an elongation at 72ºC for 1 min), finally, the primer extension was done at 72ºC for 7min.

### SSR markers

A set of six SSR primers were employed to detect polymorphism among the tested Faba bean cultivars as shown in Table (2). The PCR was done in 25 µl final reaction volume (12.5 µl Master Mix, 2.5 µl primer (10 pcmol), 3 µl template DNA (10 ng) and 7 µl $dH_2O$) as described by Khalifa *et al.* (2021).

PCR was programmed to; denaturation for 5 min at 94ºC, 40 cycles (each cycle comprised denaturation at 94ºC for 40 sec, an annealing at 55ºC for 50 sec,

and an elongation step at 72ºC for 1 min). Finally, the primer extension was done for 7min at 72ºC.

**Detection of the PCR Products**

The PCR products were checked and photographed after electrophoresis in a 1.5% agarose containing 1X TBE buffer at 100 volts.

**Data analysis**

The clear bands were scored as either present (1) or absent (0) for all samples. Then, dice's similarity matrix coeffi-cients were then calculated between genotypes using UPGMA method. The phylogenetic tree was constructed using the PAST software Version 1.91 (Hammer *et al*., 2001).

## RESULTS AND DISCUSSION

**SRAP analysis**

In this study, a set of six SRAP primers were employed to highlight the polymorphism among five of the essential Faba bean cultivars of Egypt (Giza 716, Giza 843, Sakha 4, Mariot 2, and Nubaria 5). PCR products yielded a total of 70 bands, among them 35 bands were detected for each of polymorphic and monomorphic with polymorphism percentage (50 %) as shown in Fig. (1) and Table (4). The polymorphic bands varied from 2 (SRAP-2) to 7 (SRAP-1, SRAP-3 and SRAP-6) with mean of band frequency 0.7. In parallel, the highest percentages of polymorphism were remarked with primers SRAP-3, SRAP-4, SRAP-5, SRAP-1, SRAP-6 and SRAP-2, respectively. Moreover, the highest value of similarity was registered among the cultivars (Sakha 4 and Mariot 2) with 0.87, while the lowest similarity was noted between Sakha 4 and Giza 843 with 0.75 (Table 3). Dice's similarity data were used to construct the phylogenetic tree (Fig. 2). The dendrogram separated the cultivars into two main sets, the first set comprised the cultivars (Giza 716, Sakha 4, Mariot 2 and Nubaria 5), while the second cluster involved only Giza 843 cultivar. Previous studies employed SRAP for exploring genetic diversity among several legume plants (Ariss and Vandemark, 2007; Esposito *et al*., 2007; Rana *et al*., 2009 and Castonguay *et al*., 2010). Alghamdi *et al*. (2012) examined the genetic variability among 58 faba bean genotypes using fourteen SRAP primers. SRAP markers succeeded to discriminate between the different genotypes and the Egyptian and Sudanian genotypes were grouped in one cluster. Furthermore, Elshafei *et al*. (2019) evaluated the genetic variability between eighteen genotypes of Faba bean *via* eight SRAP primers. The number of polymorphic bands for each marker ranged from 2 to 9. In parallel, Essa *et al*. (2023) employed each SCoT and SRAP for examination of genetic diversity among some Egyptian Faba bean cultivars. The SRAP marker achieved 80% polymorphism and was better than SCoT marker that exhibited 70.93% polymorphism. The studied genotypes were classified into three groups. The first group comprised Giza 716 cultivar; the second group involved Sakha 1, Sakha 3,

Sakha 4, and Akba 3300 cultivars, while the third cluster included Nubariya 1 and Nubariya 3 cultivars.

**SSR analysis**

Six SSR markers were utilized for exploiting the polymorphism among the tested Faba bean cultivars. These markers were selected due to their correlation to plant disease resistance as previously shown by Khalifa *et al*. (2021). All the tested markers exhibited clear bands; five SSR markers (KVFG460, KVFT1400, KVFT5761, KVFT906, and KVFT1913) gave two bands, while KVFG588 marker exhibited only one band. The tested SSR markers produced eleven bands in total, 27% of these bands were polymorphic (Fig. 3). In addition, the highest value of similarity was recorded among the cultivars (Giza 716 and each of Giza 843 and Sakha 4) with 0.95, while the lowest similarity was noted between the cultivars (Mariot 2 and Giza 716 or Nubaria 5 and Giza 843) with 0.82 (Table 5). The cluster analysis separated the cultivars into two main categories, the first category comprised the cultivars (Sakha 4, Mariot 2 and Nubaria 5), while the second category involved Giza 716 and Giza 843 cultivars (Fig. 4).

It was stated that the exploration of genetic diversity among Faba bean is critical factor in success of Faba bean breeding programs (Alghamdi *et al.*, 2012).

EL-Shaer and Helal (2020) assessed the genetic divergence among 18 Egyptian faba beans cultivars using SSR primers. Moreover, SSR markers were employed efficiently to discriminate and establish phylogenetic relationships among the different Faba bean genotypes (Akash *et al*., 2017 and Tahir *et al*., 2019). In this study, SSR markers succeeded to discriminate genetically among the tested Faba bean cultivars (Fig. 4). Interestingly, cultivars of Giza 716 and Giza 843 were clustered in one group and distinguished from other cultivars. These results concur with the study of Khalifa *et al*. (2021) who investigated the genetic diversity between twelve bean genotypes using 27 SSR primers. Most of primers produced one band, while eleven primers exhibited more than one band, and the cluster analysis discriminated Sakha1 and Noubaria1 cultivars from other Faba bean cultivars. The inconsistency in the cluster analysis between the SRAP and SSR markers are ordinary issue due to each marker amplify unique DNA sequences.

## SUMMARY

SRAP and SSR markers were employed efficiently to discover the genetic distinction and phylogenetic relationships among five cultivars of Egyptian Faba bean (Giza 716, Giza 843, Sakha 4, Mariot 2 and Nubaria 5). SRAP primers exhibited a total of 70 bands and 35 (50%) were polymorphic bands. All the SSR markers exhibited clear bands; five SSR markers (KVFG460, KVFT1400, KVFT5761, KVFT906, and KVFT1913) gave two bands, while KVFG588 marker produced only one band. The tested SSR markers produced eleven bands, whereas 27 % of

these bands were polymorphic. Each of SRAP and SSR markers established specific phylogenetic relationships.

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Table (1); Sequence of SRAP primers

| Primer | Forward Primer | Reverse Primer |
|---|---|---|
| SRAP-01 | me1- 5′-TGAGTCCAAACCGGATA-3′ | em1- 5′-GACTGCGTACGAATTAAT-3 |
| SRAP-02 | me1- 5′-TGAGTCCAAACCGGATA-3′ | em3- 5′-GACTGCGTACGAATTGAC-3′ |
| SRAP-03 | me1- 5′-TGAGTCCAAACCGGATA-3′ | em4- 5′-GACTGCGTACGAATTTGA-3′ |
| SRAP-04 | me2- 5′-TGAGTCCAAACCGGAGC-3′ | em1- 5′-GACTGCGTACGAATTAAT-3 |
| SRAP-05 | me2- 5′-TGAGTCCAAACCGGAGC-3′ | em2- 5′-GACTGCGTACGAATTTGC-3′ |
| SRAP-06 | me2- 5′-TGAGTCCAAACCGGAGC-3′ | em3- 5′-GACTGCGTACGAATTGAC-3′ |

Table (2): The SSR primer sequences.

| No. | Marker Name | sequence (5’ - 3) |
|---|---|---|
| 1 | KVFG460 | F- AAGCGTCAGACATGTTGATTTTTCT |
| | | R- TGCAACAACTACAACTGCAAAATTG |
| 2 | KVFT1400 | F- CATGGTTGAGATCTATTCCTGCAAC |
| | | R- TCAAGCTTGGAAATCTTCAATCACC |
| 3 | KVFG588 | F- TCTCCTTTTTCAAGCTCAATGTCAA |
| | | R- TTGTAATGGCTATGCAACAACACAT |
| 4 | KVFT5761 | F- AGTTGTTTCCTCTGATAGACAGCTT |
| | | R- AATTTCTTCAGATGAGAGTCGGGAA |
| 5 | KVFT906 | F- ATGAAATTGAGACTGTTGGGAACAC |
| | | R- AGTTGTTTCCTCTGATAGACAGCTT |
| 6 | KVFT1913 | F- AAACTCTCTCGACATTCTCCGAATC |
| | | R- ATTCCGAGAACAAGAATGTCACCTA |

Table (3). The similarity values among Faba bean cultivars based on SRAP data.

| | Mariot 2 | Giza 843 | Giza 716 | Nubaria 5 | Sakha 4 |
|---|---|---|---|---|---|
| Mariot 2 | 1.00 | | | | |
| Giza 843 | 0.82 | 1.00 | | | |
| Giza 716 | 0.86 | 0.83 | 1.00 | | |
| Nubaria 5 | 0.86 | 0.84 | 0.82 | 1.00 | |
| Sakha 4 | 0.87 | 0.75 | 0.84 | 0.85 | 1.00 |

Table (4). SRAP analysis of Faba bean cultivars.

| Primer | TB | MB | PB | % P | F | Fragment sizes |
|---|---|---|---|---|---|---|
| SRAP-1 | 15 | 8 | 7 | 47 | 0.7 | 200-1050 |
| SRAP-2 | 6 | 4 | 2 | 33 | 0.8 | 170-560 |
| SRAP-3 | 11 | 4 | 7 | 64 | 0.7 | 250-750 |
| SRAP-4 | 10 | 4 | 6 | 60 | 0.7 | 140-1000 |
| SRAP-5 | 12 | 6 | 6 | 50 | 0.7 | 180-1150 |
| SRAP-6 | 16 | 9 | 7 | 44 | 0.8 | 160-770 |
| Total | 70 | 35 | 35 | 50 | | |

(TB) Total Number of Bands, (MB) Monomorphic Bands, (PB) Polymorphic Bands, (%P) Percentage of Polymorphism, (F) Mean of band frequency.

Table (5). The similarity values among the tested Faba bean cultivars as revealed by SSR data.

| | Mariot 2 | Giza 843 | Giza 716 | Nubaria 5 | Sakha 4 |
|---|---|---|---|---|---|
| Mariot 2 | 1.00 | | | | |
| Giza 843 | 0.88 | 1.00 | | | |
| Giza 716 | 0.82 | 0.95 | 1.00 | | |
| Nubaria 5 | 0.93 | 0.82 | 0.89 | 1.00 | |
| Sakha 4 | 0.88 | 0.89 | 0.95 | 0.94 | 1.00 |

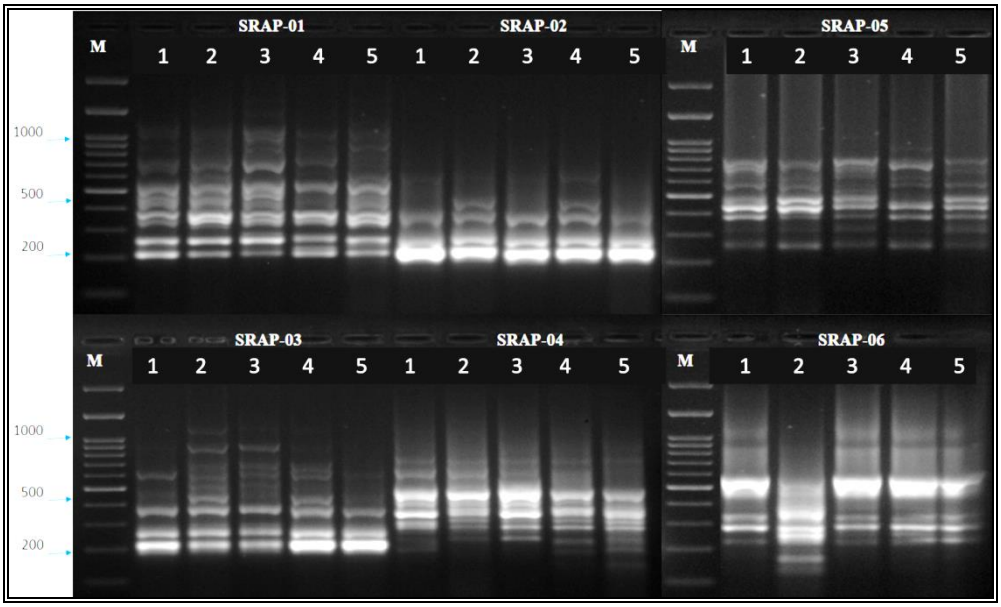


Fig. (1). SRAP fingerprinting of Faba bean cultivars: M; DNA marker, lanes 1-5; Mariot 2, Giza 843, Giza 716, Nubaria 5, and Sakha 4, respectively.

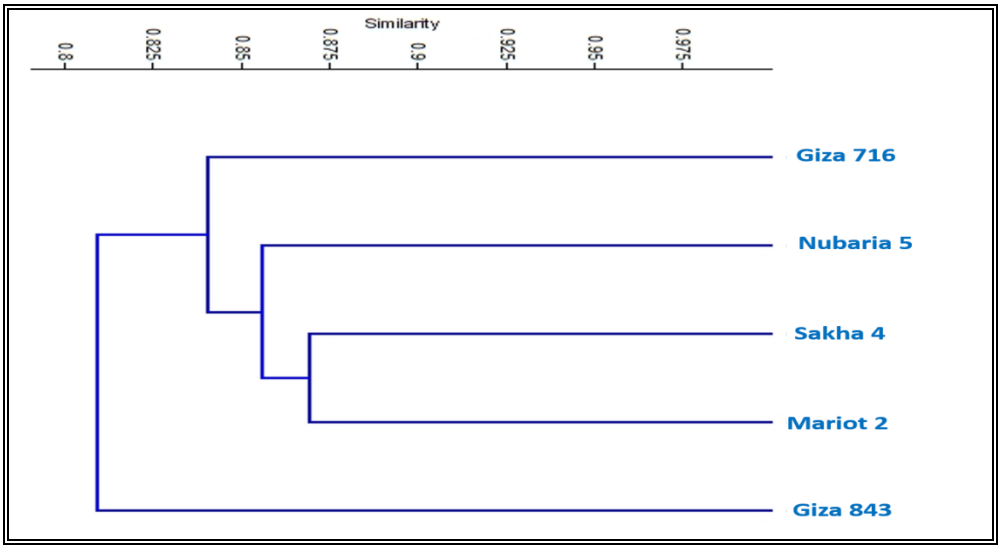


Fig. (2). Phylogenetic tree of Faba bean cultivars as revealed by SRAP data and performed using the PAST software.

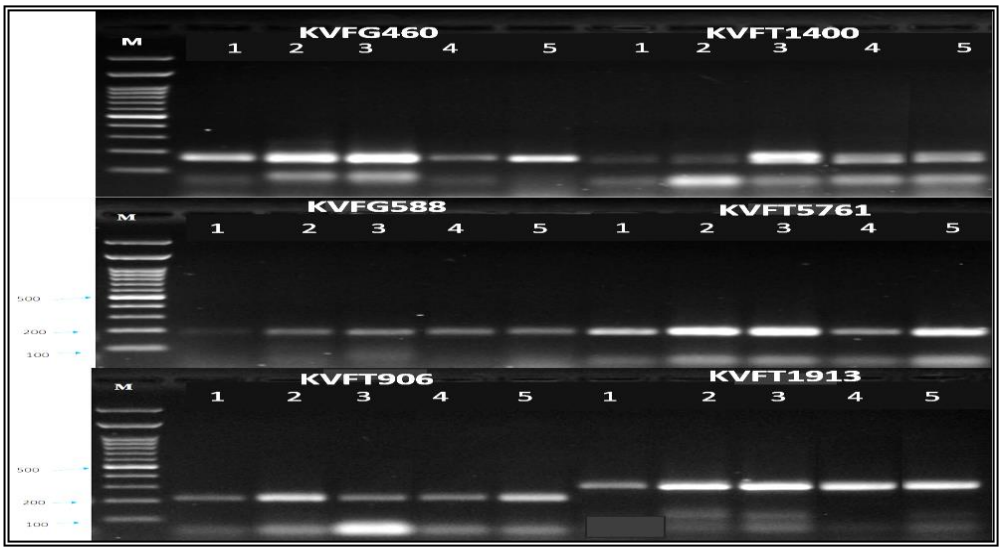


Fig. (3). SSR fingerprinting of Faba bean cultivars: M; DNA marker, lanes 1-5; Mariot 2, Giza 843, Giza 716, Nubaria 5, and Sakha 4, respectively.

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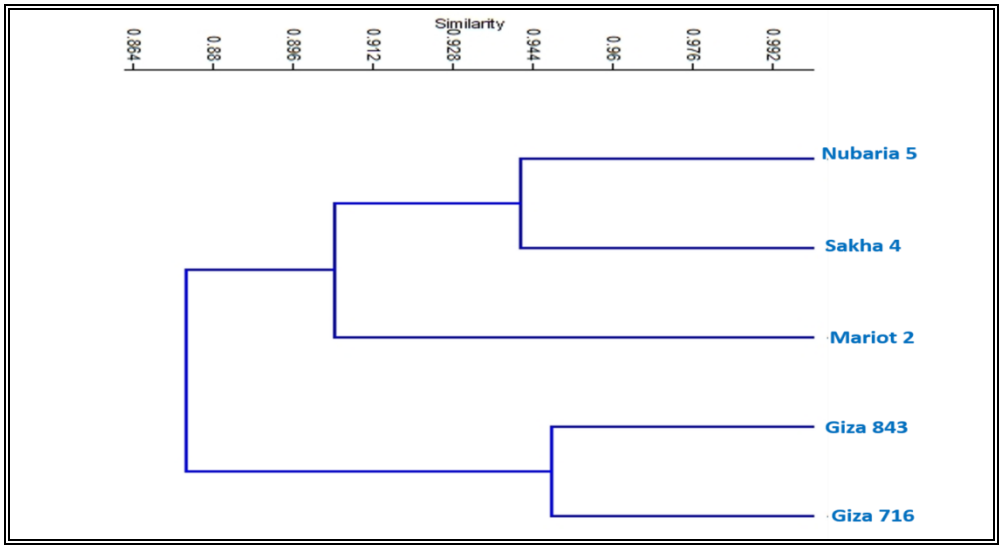


Fig. (4). Phylogenetic tree of Faba bean cultivars as revealed by SSR data and performed using the PAST software.