

## Genetic diversity on *Vicia faba* by using SSRs

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### Abstract

The goal of this research was to establish a new genetic database of faba bean SSR primers and categories them according to their target genes and biological processes. Twenty-seven SSR PCR primers were synthesized to 12 Egyptian faba bean genotypes. Approximately 11 SSR provided one to two PCR bands, whereas other SSRs provided only one sharp band with polymorphic band size. There were 13 polymorphic primers. The polymorphism information content was 0.3, which implied moderate informativeness. The principle component analysis (PCA) was used to infer the genetic structure of the studied faba accessions using the data of applied SSR primers. The PCA analysis revealed 24.3% and 22.1% explanation of the genetic variation on both axes. The heatmap conducted using SSR data and clustered the faba beans into three groups, where Noubaria 1, Noubaria 2, Sakha 1 and Giza 3 were differentiated by their high genetic variation compared to other accessions.

**Keywords:** SSR. Database. PCR. Egypt. Faba bean. Genetic diversity

### Introduction

(*Vicia faba* L.) is a prospective candidate crop for providing protein and starch for human meals and animal feed in many nations, including China, Ethiopia, Egypt, France, and Australia. The Faba bean is a diploid species with  $2n = 12$  chromosomes and one of the largest genomes (about 13,000 Mb) among crop legumes (El-Rodeny et al. 2014).

Egypt produced 287575 tons of *V. faba* in 2017, up from 27290 tons in 2016 (FAO). Due to the difficulty of managing hybridization on a large scale, most faba bean lines have been cultivated from inbred lines despite repeated recommendations for developing relatively heterogeneous synthetic varieties. As a result, the geographic distribution and goals of this species' breeding programs follow where the largest consumption occurs. As opposed to soybeans, faba bean selection programs are rare and tiny in comparison to the size of the market. They rely on gene banks for genitors and alleles of interest in part (Duc et al., 2010). However, because production has decreased noticeably in recent decades, owing primarily to biotic and abiotic stress, to accomplish so, it is vital to produce materials that reduce side effects.

Characterization, both agromorphological and molecular, is required to learn the degree of diversity and relationship between accessions. Salazar-Laureles et al (Salazar-Laureles et al., 2015). Only

using morphological/agronomic variables to build gene pools is insufficient because these parameters are either influenced by environmental factors and plant growth stage or reflect only limited variation. Plant scientists now have more tools to analyses germplasm and determine genetic diversity and population structure thanks to the rapid development of molecular markers ( Terzopoulos and Bebeli 2008). In this context, it is necessary to characterize genetic variation in current local and Mediterranean faba bean germplasm supplies in order to improve agricultural yields and impart biotic and abiotic stress resistance. Molecular markers have generally been used in the *Vicia faba* to examine genetic variety at the interspecific level; nevertheless, knowledge about genetic variability within elite faba bean germplasm is insufficient for breeding purposes. Breeders can only benefit from genetic variation within the species because *Vicia faba* cannot be effectively crossed with any other species (Link et al., 1995).

As it has in many other crop species, marker assisted selection (MAS) is predicted to transform faba bean breeding tactics. The integration of MAS into faba bean breeding programs has been hindered due to the huge genome size and a lack of genomic tools (Khalifa et al. 2021; Duc et al. 2010).

In *Vicia* species and *V. faba* accessions, DNA marker technology like as RFLP and AFLP has been widely used to examine genetic diversity and construct genetic linkage maps for this purpose.

Because they combine the reliability of RFLPs with the power of PCR and the flexibility to analyse several loci in a single experiment, AFLPs have shown to be quite useful in *V. faba* research. To avoid selection and genetic drift in the gene bank collection, intraaccession diversity in barley germplasm was evaluated using AFLP (Gresta et al, 2010).

SSRs, also known as microsatellite repeats, could be utilised to assess faba bean diversity, phylogenetic relationships, and genetic structure (Tufan and Erdoğan 2017). Microsatellites showed increased effectiveness in genetic diversity analysis, revealing substantial allelic diversity, because they are co-dominant and plentiful in the plant's genome. Microsatellites also have a high degree of transferability to clusters that are closely related (El-Esawi 2017).

As a result, the purpose of this research was to develop genomic markers based on faba bean genomic and transcriptome data that could be useful for tracing and optimizing faba genetic resources. The purpose is to construct, classify, and annotate a new genomic database of SSR PCR primers to target genes and cell processes. In addition, the genetic structure of several selected faba bean cultivars, as

well as the diversity and efficacy of microsatellite markers applied to faba bean, will be investigated in this study.

### Materials and methods

Molecular Genetics and Genome Mapping Laboratory (MGGM) - Genome Mapping Department of the Agriculture Genetic Engineering Research Institute (AGERI), Agriculture Research Center (ARC), Giza, Egypt, conducted this study from 2018 to 2021.

#### Materials:

This study included twelve Faba bean (*Vicia faba* L) cultivars, i.e. "Giza-716, Giza-843, Giza-40, and Giza-3, Wady-1, Masr-1, Nobuaria-3, Nobuaria-2, Nobuaria-1, Sakha-1, Sakha-3, Sakha-4". These cultivars were released in Field Crops Research Institute, ARC, Egypt.

#### Methods:

For lab validation, twenty-seven SSR primers were chosen (table 1). These primers are linked to the NBS-LRR and F box gene families, which are involved in disease resistance and plant development (Khalifa et al. 2021)

**Table 1:** selected SSR PCR primers

Name	Forward sequence	Reverse sequence
KVFG128	CTCTCTGCCAAAACATGTTGTAGT	TAGCAGGGATTGAAAGAGACTTCAA
2	AACCTTAACCACCACCAAAGATTTT	GAAACCAAAGAGAG-
KVFG136	CCGCCGTATCAAATTCGAATTCATA	GACTTTCAAGG
0	CCGTTTCAAACCCTAAACCTTCAA	GTTGAATTGATGAAAACGGGTTTGG
KVFG222	AAGCGTCAGACATGTTGATTTTTCT	CCAATTCAAAGGCTTAGAATGTGGT
KVFG271	TCTCCTTTTTCAAGCTCAATGTCAA	TGCAACAACACTACAACACTGCAAATTG
KVFG460	GCATATTCCTGTTCTTGTAGAAGGC	TTGTAATGGCTATGCAACAACACAT
KVFG588	ATTGCGATAATTTGGTCTTCCTTCC	GGGTG-
KVFG606	CATGGTTGAGATCTATTCCTGCAAC	GATTGAAAGGAAAAATCGAA
KVFT1093	AAACTCTCTCGACATTCTCCGAATC	TGTCAATTGAAAATCGCTCGAGTTT
KVFT1400	GAAACCAAAGAGAGGACTTTCAAGG	TCAAGCTTGGAAATCTTCAATCACC
KVFT1913	CCAATTCAAAGGCTTAGAATGTGGT	ATTCCGAGAACAAGAATGTCACCTA
KVFT1992	GTTTGTGACGGAAGAGAGTAAGTG	AACCTTAACCACCACCAAAGATTTT
KVFT2303	AAGGTTGAAAGAGTGGAAGTGTTG	ACCCTAAACCTTCAACTAGTTCAAA
KVFT29	ACAAAGCATGAGAACACATTAGCAA	CCCATACAAAGCATGAGAACACATT
KVFT3141	TGGCACTAGCACCTTATGAACTAAT	AACCTTAACCACCACCAAAGATTTT
KVFT37	TTGTGAAAAGCGGTTGTTACTAGTC	GTTTGTGACGGAAGAGAGTAAGTG
KVFT4014	GCATATTCCTGTTCTTGTAGAAGGC	CGGTTGAGAATGTGAGTGTTTGTTA
KVFT4352	AGTTGATGAGGATGGAGCTAAAGTT	CTCAAACCTTATCCCTGATATTCCT
KVFT4784	TGGTTTCTCCAGAAATAGCAGTAGA	GGGTG-
KVFT49	AGTTGTTTCTCTGATAGACAGCTT	GATTGAAAGGAAAAATCGAA
KVFT541	TTGAGAGATAGCATGACTAAAGCGT	TGAAACAAAATCCTCACACCCTTA
KVFT5761		AGTTTCATCTTACCAAGCACAATG

KVFT6054	CTCCTCCCTCTTGAGAACTAGAAATG	AATTTCTTCAGATGAGAGTCGGGAA
KVFT6262	GGCCCATGTACCTCAAAGTTTTATC	AAACACCACCTTCTTCTTCTTCTT
KVFT6415	TGGTTGAGGATTTTAAGGTCGTTTG	AAGATGATTGAGGATATCAGGGTGG
KVFT809	TGGTGAAGAAGAGAAGAA-	ATTGCGATAATTTGGTCTTCCTTCC
KVFT834	GATGGAG	GTCCTTTCCCATTTTCATCAAACACT
KVFT906	ATGAAATTGAGACTGTTGGGAACAC	TGAGACACTCTCTAACCTTGGATTT
		AGTTGTTTCTCTGATAGACAGCTT

#### DNA isolation:

The DNA was extracted from fresh leaves of twelve faba bean varieties released in Egypt, according to a CTAB modification protocol. A one-hundred-milligram leaf was grinded by Liquid nitrogen, a mortar, and a pestle were used. 700µl of a CTAB buffer (preheated at 65°C) was added. samples were placed in 1.5-ml tubes and thoroughly mixed. The samples were incubated for (60 min) at 65 °C in water baths with a shaker, then for 10 min in ice. 700 µl chloroform: Isoamyl alcohol(24:1) was added to the samples and thoroughly mixed for 20 minutes. The samples were centrifuged for 30 minutes at 13,000 rpm, then the top phase was transferred to new 1.5-ml tubes. 70 µl sodium acetate and 700 µl isopropanol were added to the samples and incubated at 20 °C for 2 hours. The samples were centrifuged at 13,000 rpm for 5 minutes before being discarded. About 500 µl of 70% ethanol was added to the samples, centrifuged at 13,000 rpm for 5 minutes, and the top phase was removed. In 50 µl of TE buffer, the DNA strands were dissolved.

The PCR conditions for primer subset validation were as follows: an initial denaturation cycle at 95 °C for 5 min; followed by 35 cycles of 95 °C for 30 s, the annealing temperature was adjusted for each primer pair separately for 30 s, 72 °C for 30 s, and the final extension step at 72 °C for 7 min.

#### Molecular marker and phylogenetic analyses

Gel Documentation System (BIO-RAD 2000) was used to view and document SSR PCR products on UV light. Clear and unambiguous PCR bands SSR

molecular analysis as 1 (present) or 0 (absent) for all faba DNA samples. Power Marker software (Liu and Muse 2005) was used to calculate the Dice coefficients of the matrix of similarities among varieties using the unweighted pair group method with arithmetic averages (UPGMA) procedure. The analysis results were used for phylogenetic tree analysis. The polymorphism information content (PIC) was performed to identify the capacity of the SSR marker to detect genetic variation among faba varieties using Power Marker software (Liu and Muse 2005).

#### Results

##### Validation of PCR primers and molecular analysis:

The NBS-LRR and F box gene families are connected to twenty-seven SSR PCR primers. All of the SSR primers worked well and produced scoreable PCR bands. Thirteen SSR primers out of the total of 27 were polymorphic (48 percent). Eleven SSR primers produced one to two PCR bands, but others only produced one strong band with a variable band width (Fig 1). Similarly, Kaur et al. (2012) screened a subset of 96 ES T-SSR PCR primers designed from faba bean transcriptomic samples, 84% showed effective amplification and 29.6% showed polymorphic PCR bands. In addition, 102 SSR-designed primers out of 105 were reproducible, 94 of which were polymorphic in earlier studies (Yang et al. 2012) and others were able to amplify 31 primer pairs out of 34 in prior studies.

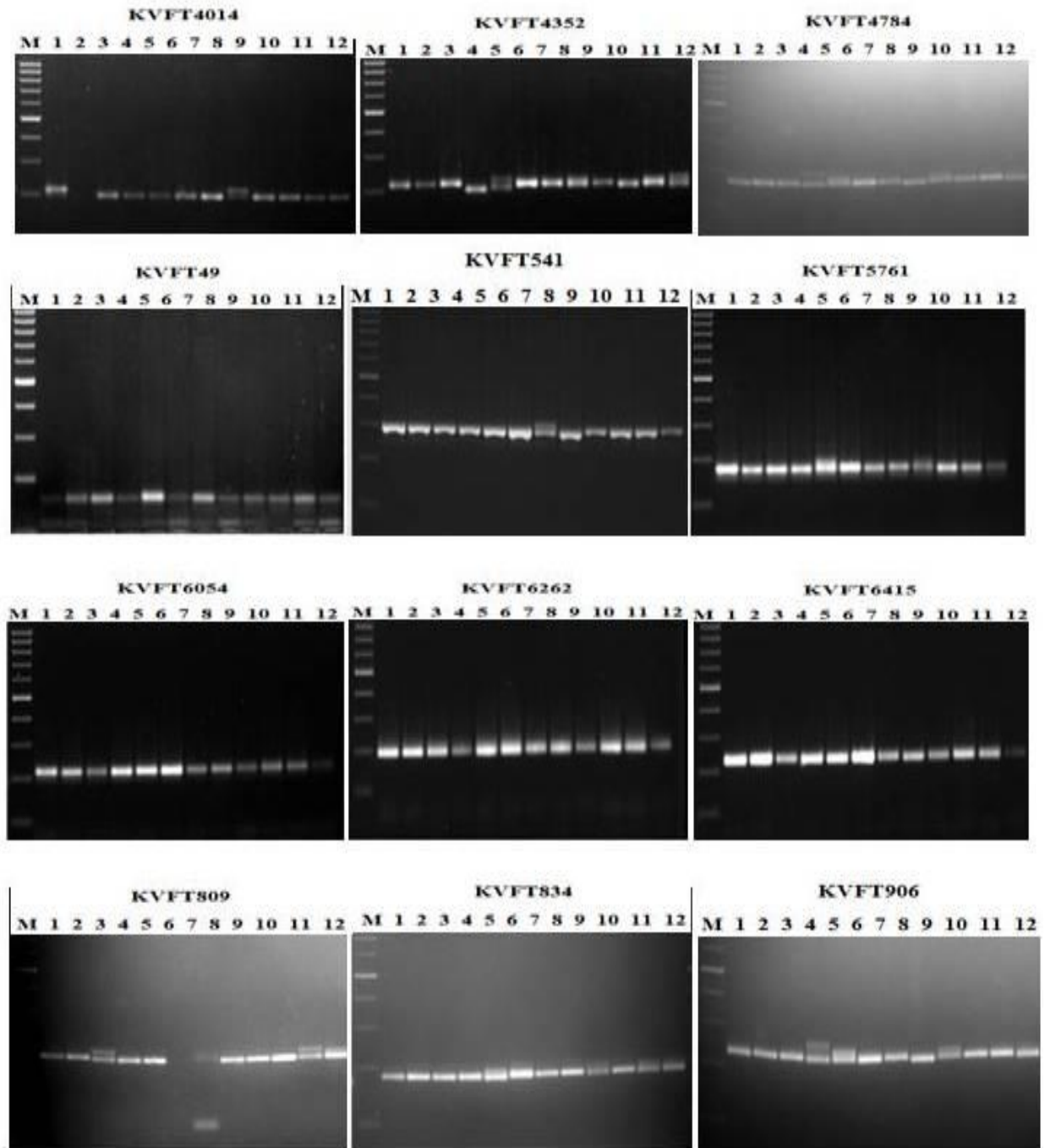
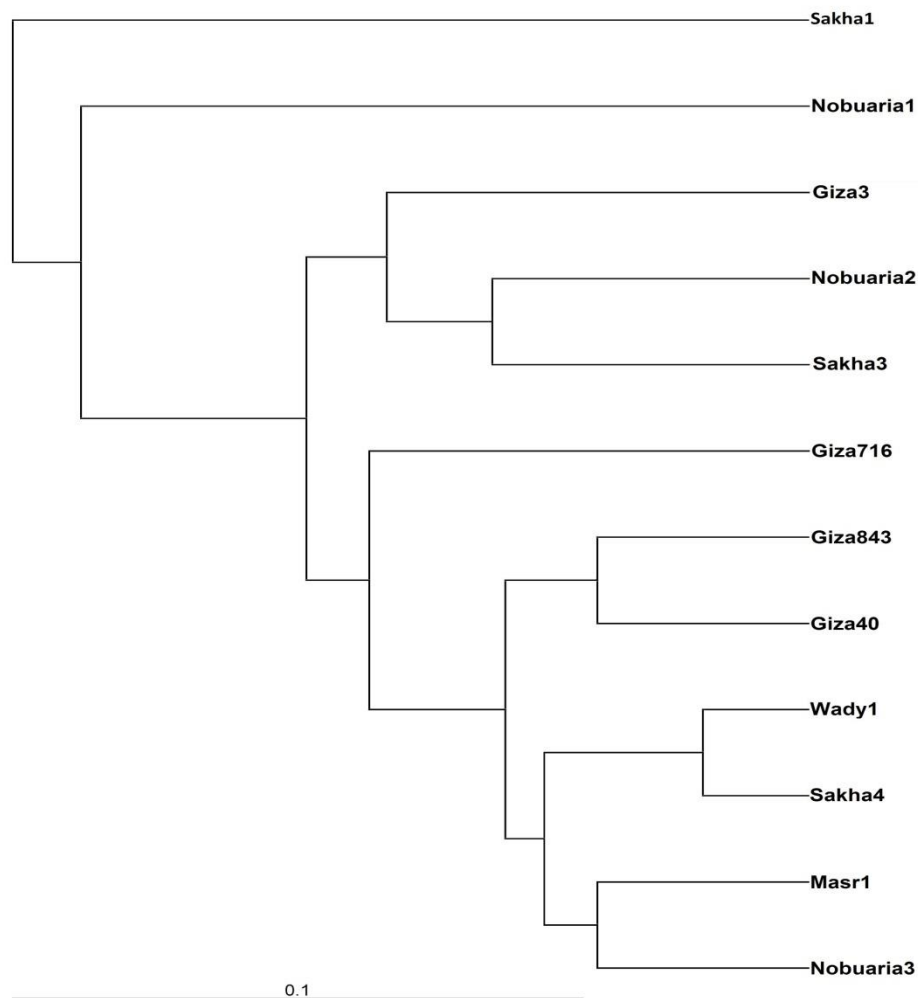


Figure1: PCR profiles of the SSR assay applied to studied faba accessions.

The information content of polymorphism (PIC) is a metric that assesses a marker's ability to detect genetic variation in the diversity group under investigation. Furthermore, marker information levels range from 0 to 1, with PICs larger than 0.5 being extremely informative and PICs between 0.5 and 0.25 being somewhat informative (Botstein et al., 1980). The PIC values for SSRs ranged from 0 to 0.57. Our average PIC score would be 0.3 if we

ignore the monomorphic indications (which have a PIC value of 0), signifying weak informativeness. The SSR-based phylogenetic tree (Figure 2) shows three distinct clusters, with Sakha 1 and Nobuaria 1 separated into two. The remaining genotypes were separated into three sub-clusters: Nubaria 3, Giza 3, and Sakha 3 in one cluster, and Giza 716 in another, and while the rest in a third.



**Figure 2:** The phylogenetic tree of the twelve Egyptian faba bean genotypes

The principle component analysis (PCA) was used to infer the genetic structure of the studied faba accessions using the data of applied SSR primers (Figure 3). The PCA analysis revealed 24.3% and 22.1% explanation of the genetic variation on both axes. Accession of Noubaria1, Noubaria2 and Sakha1 were separated from the main group. The

heatmap conducted using SSR data is shown in (Figure 4). The heatmap clustered the faba beans into three groups, where Noubaria 1, Noubaria 2, Sakha 1 and Giza 3 were differentiated by their high genetic variation compared to other accessions.

Figure 3: The principle component analysis of the studied faba accessions.

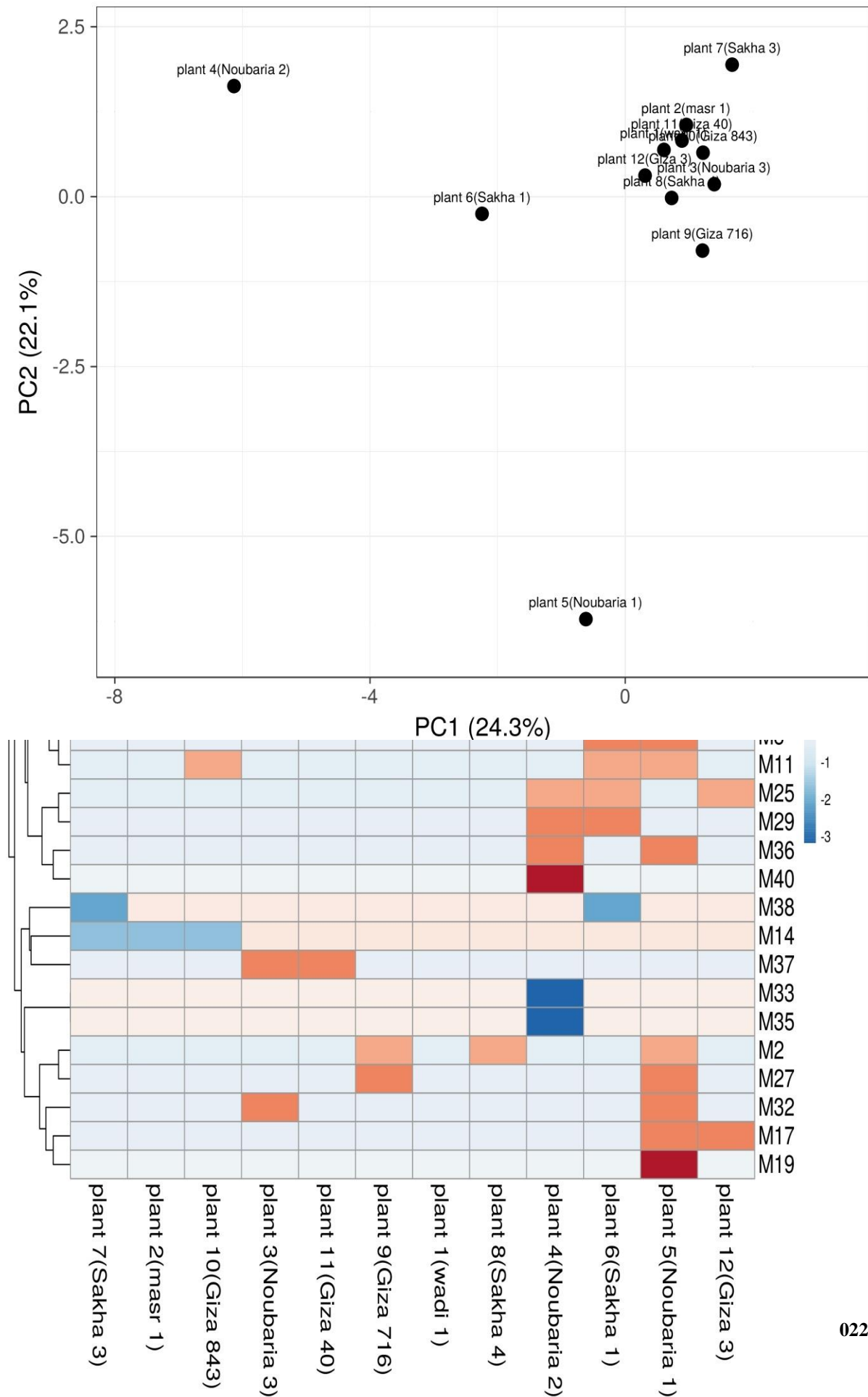


Figure 4: The heatmap of the data of the SSR data.

## Discussion

Twenty-seven SSR PCR primers have been linked to genes involved in plant development and resistance, including the NBS-LRR and F box gene families. All SSR primers were successfully used and resulted in PCR bands that could be scored. Thirteen of the twenty-seven SSR primers tested were polymorphic (48 percent), Kaur et al. (2012) assessed a subset of 96 EST-SSR PCR primers generated from faba bean transcriptome data and found that 84 percent indicated effective amplification and 29.6% displayed polymorphism. Furthermore, 102 out of 105 SSR-designed primers were reproducible, with 94 of them being polymorphic in earlier research (Yang et al., 2012), and others able to amplify 31 out of 34 primer pairs in previous studies (Akash et al., 2012).

The average polymorphism percentage for SSR primers developed for chickpea, flax, lettuce, and *Lilium* was 52.94 percent, 24.4 percent, 28.88 percent, and 41 percent, respectively (Asadi et al., 2020; Pan et al., 2020; Wang et al., 2017; Biswas et al., 2020). Using verified SSR primers, we found moderate PIC values ranging from 0.14 to 0.57, with an average of 0.3 for polymorphic primers. This could indicate a low amount of genetic usefulness. A phylogenetic tree based on genetic binary data from certified SSR markers can show the genetic differentiation amongst faba cultivars. Other faba bean cultivars vary greatly from Sakha1 and Noubaria1. Due to their salty soil tolerance, these native Egyptian cultivars have attracted interest in recent years (Amer et al., 2018). There were three categories for the remaining faba bean cultivars. SSR analysis was used to look at genetic variation in a number of faba bean types from different parts of the world. El-Esawi et al., (2017) used SSRs to look at the genetic diversity of 35 faba bean genotypes from Eastern Africa, Northern Africa, and the Middle East. He chose 12 Egyptian faba bean varieties, the bulk of which were clustered together (two sub-groups), with PICs ranging from 0.44 to 0.78 an average of 0.58. Mahmoud and El-Fatah (2020) used SSR primers to study the genetic diversity of numerous Egyptian faba bean cultivars. SSR and other molecular tests were used to generate a phylogenetic tree that divided faba beans into three groups.

PCA was helpful in identifying the genetic structure of the tested faba accessions using SSR assay (Figure 3). The PCA analysis revealed 24.3% and 22.1% explanation of the genetic variation on both axes. This indicated the powerful identification ability of SSR technology to explain the detected genetic variation across the studied faba set. Accession of Noubaria 1, Noubaria 2 and Sakha 1 were separated from the main group, which could be related to the

geographical distance between both collected locations. The heatmap was useful in showing the scale of genetic variation across the studied accessions, where genetic structure could be illustrated depending on the genetic variation (Figure 4). Both heatmap and PCA analyses were inconsistent with each other, where Noubaria 1, Noubaria 2, Sakha 1 and Giza 3 were differentiated by their high genetic variation compared to other accessions. Indicating that the genetic variability of these accessions is valid and it could help future breeding programs.

## Conclusions

The SSR assay is very repeatable and can reveal important details about faba bean genetic diversity and structure. These markers are located near hundreds of faba bean genes that may influence crucial morphological and agronomic features such as disease resistance and stress tolerance. The genetic variety and relationship of local Egyptian faba bean genotypes were investigated in order to validate dozens of SSR markers, which produced good polymorphism and informativeness while demonstrating their genetic diversity and relationships. These markers could be valuable in determining the genetic resources of local faba beans.

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### التنوع الوراثي في الفول البلدي باستخدام التتابعات القصيرة المتكررة SSR

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كان الهدف من هذا البحث إنشاء قاعدة بيانات وراثية جديدة لبادئات الفول البلدي للتتابعات القصيرة المتكررة SSR وتصنيفها حسب الجينات المستهدفة والعمليات البيولوجية. سبعة وعشرون بادئة SSR PCR تم انشائها و تطبيقها علي 12 صنف من الفول البلدي المصري. قدم ما يقرب من 11 SSR حزمة واحدة إلى حزمتين PCR ، بينما قدمت SSRs الأخرى حزمة دقيقة واحدة متعدد الأشكال. كان هناك 13 بادئة متعددة الأشكال. كان محتوى معلومات تعدد الأشكال 0.3 ، مما يعني وجود معلومات معتدلة. تم استخدام تحليل المكون الأساسي (PCA) لاستنتاج التركيب الجيني لاصناف الفول البلدي تحت الدراسة باستخدام بيانات بادئات SSR المطبقة. كشف تحليل PCA عن تفسير 24.3% و 22.1% للاختلاف الجيني على كلا المحورين. تم إجراء heatmap باستخدام بيانات SSR وقسمت الفول إلى ثلاث مجموعات ، حيث تميزت النوبارية 1 ، والنوبارية 2 ، وسخا 1 ، وجيزة 3 باختلافها الوراثي العالي مقارنة لاصناف الأخرى.