GROWTH, YIELD PARAMETERS AND GENETIC DIVERSITY AMONG SOME EGYPTIAN FABA BEAN (*Vicia faba* L.) CULTIVARS USING SCOT AND ITS MARKERS ANALYSES

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Faba bean (*Vicia faba* L.; 2n=12) is among the most widely cultivated pulses in Egypt and rank as one of the oldest crops grown globally (Bakry *et al.*, 2011 and Zong *et al.*, 2009). It have been cultivated since around 3000 B.C. in Ancient Egypt, faba beans have been integral to various agricultural systems due to their nutritional and economic significance (Duc *et al.*, 2010). Faba beans are consumed for both their green pods and dried seeds (Duc *et al.*, 2010). Due to their high protein content, legumes are also viewed as crucial for transitioning to more sustainable diets, with the potential to replace animal-based protein sources (Willett *et al.*, 2019).

However, the global legume market is dominated by a few crops, such as soybeans (*Glycine max*), which account for 80% of total legume production (FAOSTAT, 2021). Diversifying legume cultivation is therefore essential. As a legume with seeds containing about 30% protein, faba beans have considerable potential for wider use in food production. Nevertheless, issues like yield instability, vulnerability to diseases, and the presence of antinutritional factors such as tannins and compounds that cause favism hinder their widespread adoption (Maalouf *et al.*, 2018).

Strengthening breeding programs and enhancing germplasm characterization are crucial steps toward fully realizing the potential of faba beans as a sustainable crop (Duc *et al.*, 2010 and Ellwood *et al.*, 2008). The Egyptian faba

bean population is characterized by a great phenotypic variation in growth, seed yield, and seed quality traits. There are early and late maturing varieties. Also the Egyptian faba bean population contains tolerant to fungal diseases and insect infestations genotypes, and others that are moderately tolerant or sensitive (Abo-Hegazy and Darwish, 2022). The great phenotypic variation among the Egypt faba bean varieties is due to the great genetic diversity between these varieties in terms of pedigree and country of origin of the parents of these varieties (Soliman *et al.*, 2024).

Many studies have investigated the effect of the environment on the phenotypic variation of faba beans, while few studies have sought to estimate the genetic variation and the extent of its effect on the phenotypic variation of faba beans. Recent advancements in molecular genetics now allow for the quantification of genetic diversity at the DNA level through the detection of various molecular markers (Abid *et al.*, 2015). One key advantage of molecular markers is their immunity to influence plant developmental stages or environmental factors (Abdel-Razzak *et al.*, 2012).

Various molecular marker techniques, such as RFLP, RAPD, AFLP, TRAP, and SSAP, have been used to evaluate genetic variations among *V. faba* genotypes (Abid *et al.*, 2015). Recently, Collard and Mackill (2009) introduced a rapid and innovative DNA marker technology known as start codon targeted (SCoT) polymorphism. This marker was designed based on a short, conserved region flanking the Adenine-Thymine-Guanine (ATG) start codon in plant genes. SCoT markers resemble RAPD and ISSR techniques in that a single primer serves as both forward and reverse. Additionally, as a PCR-based gene-targeting method, SCoT analysis is cost-effective and efficient (Bhattacharyya *et al*., 2013). Numerous studies indicate that SCoT may be more effective than other dominant DNA molecular markers, such as RAPD and ISSR, due to its gene-targeted nature (Gupta *et al.*, 2018). SCoT also outperforms these markers in terms of higher polymorphism and improved marker resolvability (Gorji *et al.*, 2011). Furthermore, SCoT can produce co-dominant markers resulting from insertions and deletions, as well as dominant markers caused by sequence variations, similar to RAPD and ISSR (Aswathy *et al.*, 2017).

Ribosomal DNA has been shown to be a valuable region for study (Ward, 1994) and is applicable to any organism. It includes regions such as the 5.8S, 18S, and 28S rDNA genes (Kularatne *et al.*, 2004), which exhibit relatively few differences between species, as well as the internal transcribed spacer (ITS) regions between genes, which are significantly more variable (Ward and Adams, 1998 and Pramateftaki *et al*., 2000). Consequently, the determined ribosomal DNA sequences were also utilized to design PCR primers for these applications (Ward and Adams, 1998). Additionally, ITS re-

gions have been suggested as useful for inferring phylogenetic relationships among closely related organisms (Gurtler and Stanisich, 1996). Therefore, the main objectives of this investigation are to evaluate growth, yield and quality parameters as well as, molecular variation among seven Egyptian faba bean (Nubaria 1, 2, 5, Sakha 4, Giza 716, Giza 843 and Wady 1) cultivars using ITS sequencing and SCoT molecular markers to determine the potential genetic variations which in true provide the plant breeder with promising material that help in Faba bean improving programs.

MATERIALS AND METHODS

1. Plant materials

Seven faba beans cultivars (Nubaria 1, 2 and 5, Sakha 4, Giza 716, 843 and Wady 1) were obtained kindly from Food Legumes Research Department, Field Crops Research Institute, Agriculture Research Centre. Name, pedigree and origin of the seven tested faba bean genotypes are presented in Table (1).

2. The field experiment

The seven tested faba bean cultivars were evaluated under clay soil condition in the experimental farm of Etay El-Baroud Agriculture Research Station-Beheira Governorate during 2022/2023 and 2023/2024 seasons in an experiment designed in randomized complete blocks design with three replications. In both seasons of the study faba bean cultivars were sown in 15th November in dry soil in plots. Each plot consists of five ridges each ridge was 4 meter long and 70 cm apart. Seeds were sown on both side of the ridge with one seed per hill and 30cm hills space. Faba bean plants were received all culture practices (weed control, fertilization treatment, irrigation and pests control) as recommended in the time and rates).

2.1. Data recorded.

Germination percentage was determined after full emergence according to the following equation:

$$\textbf{\textit{Seed germination}}\ \% = \frac{A}{B}$$ %Where:

A = *number of full establish seedlings per plot*

B= *number of seeds that sowing in the same plot*

Number of days to maturity: was recorded as the number of days to reach 95 % maturity of whole plant in the plot.

At harvest day, 10 guarded plants chosen randomly were taken from each plot to determine plant height (cm), number of branches/plant, number of pods/plant, 100-seeds weight (g), seed yield/plant (g) and seed yield/fedan (tons).

Seed yield per feddan was calculated by convert seed yield/plot (kg) to feddan (tons).

Determination of total carbohydrate and protein in dried seeds:

Total carbohydrate was determined using phenol sulphuric method (Dubois *et al*., 1956).

Total nitrogen percentage was determined by Modified Micro-Kjeldahl method as described by AOAC (1988) and the percentage of protein was calculated by multiplying total N values by 6.25.

2.2. Statistical analysis

Data were subjected to One-way ANOVA as the method described by Gomez and Gomez (1984) followed by compared means using least significant difference (LSD) tested at 5% level of probability using the statistical software SPSS 16 (version 4).

3. Molecular evaluation

3.1. Extraction of DNA

Genomic DNA was extracted from fresh leaves of germinated faba bean seeds using a modified version of the Doyle and Doyle (1990) method. One gram of finely ground leaf tissue was powdered in liquid nitrogen. Subsequently, 500 mg of the powdered material was transferred to 2 mL Eppendorf tubes. To each tube, 1 mL of freshly preheated 2.5 X CTAB solutions and 0.8 g of polyvinylpolypyrrolidone (PVP) were added. The mixture was incubated in a water bath at 65°C for one hour, with gentle stirring every 10 minutes. After completing the isolation and purification steps, the DNA concentration and purity were assessed using spectrophotometric analysis, following the protocol of Sambrook *et al.* (1989).

3.2. PCR Conditions for SCoT Analysis

The SCoT analysis was conducted using seven SCoT primers (Table 2). The PCR protocol included an initial denaturation at 94°C for 5 minutes, followed by 40 cycles of denaturation at 94°C for 40 seconds, annealing at 50°C for 50 seconds, and extension at 72°C for 1 minute. A final elongation step was carried out at 72°C for 7 minutes. The amplified products were verified by electrophoresis on 2% agarose gels stained with ethidium bromide, with sizes estimated using a 100 bp DNA ladder. DNA fragment sizes were then analyzed using GelAnalyzer Version three, (2007).

3.3. PCR Amplification of ITS Region

The PCR reaction was carried out in a total volume of 25 µl, which comprised 12.5 µl of 2X master mix (containing 0.05 units/µl Taq DNA polymerase in 2X PCR buffer with 4 mM MgC_{l2} and 0.4 mM of each of the four dNTPs), 10 µM of each primer [forward primer ITS-p5 (18S: 5' – CCTTATCAYTTAGAGGAAGGAG–3') and reverse primer ITS-u4 (5.8S: 5' – GCGTTCAAAGAYTCGATGRTTC– 3')], and 1 ng/µl of DNA template. The amplification protocol involved an initial denaturation at 94°C for 4 minutes, followed by 35 cycles of 45 seconds at 94°C, 40 seconds at 55°C (or 58°C), and 1 minute at 72°C, with a final elongation step

of 10 minutes at 72°C. The PCR products were then assessed by electrophoresis on a 2% agarose gel stained with ethidium bromide, and visualized using an ultraviolet transilluminator.

3.4. DNA Sequencing

Sequencing of the PCR products from the seven cultivars of faba bean was performed directionally using the amplification primers. This sequencing was carried out by GATC Biotech Ltd. at The London Bio-Science Innovation Centre in London, United Kingdom, using the ABI 3730xl DNA Sequencer.

RESULTS AND DISCUSSION

1. Comparison of growth, seed yield and seed quality of some Egyptian faba bean cultivars during 2023 and 2024 growing seasons.

1.1. Growth traits

The presented results in Table (3) confirmed highly significant diversity among all tested faba bean genotypes in all growth measurements during both studied seasons. The highest emergence percentages were recorded in Giza 843 (96.81 and 96.52%) followed by Sakha 4 (95.60 and 95.34%) and then Wady 1 (94.99 and 96.52%) during both seasons, respectively. The high seed emergence almost associated with small and medium seed genotypes while seed emergence reduce partially in large seed genotypes such as Nubaria 1, 2 and 5 as well as Giza 716. Respect to maturity date, Giza 716 was the earliest among all tested cultivars where it had the lowest number of days to maturity (136.80 and 134.38 days) followed by Sakha 4 (140.00 and 141.67 days) in the first and second seasons, respectively. On the other side, Nubaria 1 recorded the longest maturity date (154.46 and 150.67 days) in both seasons, respectively compared with all tested genotypes. Also, Giza 716 recorded the highest, plant height (110.26 and 115.71 cm) and number of branches/plant (4.58 and 5.02) in the two seasons of the study, respectively.

The diversity among Egyptian faba bean cultivars in growth traits were reported before by Abo-Hegazy, and Darwish (2022), Abdelaal, (2023) and Soliman, *et al,* (2024). In the study of Tawfik *et al*, (2018) Nubaria 1 exceeded Giza 3 and Misr 1 in all growth traits. While Kandil, *et al.* (2011) and El-Karamity *et al.* (2017) showed that both Giza 716 and Misr 1 recorded high growth values compared with others genotypes.

1.2. Yield traits

The obtained data in Table (4) revealed highly significant variation among all tested faba bean genotypes in all yield and yield components traits in both seasons. Giza 716 had the highest pods number/plant (24.79 and 24.67), seed yield/plant (63.175 and 64.099 g) and seed yield/fed (1.932 and 1.976 tons) while the highest 100-seed weight were recorded in Nubaria 1 (103.21 and 102.82 g) in both seasons, respectively. Nubaria 1 and Nubaria 5 also showed excellent yield

and yield components values. In contrast, Giza 843 had the lowest pods number/plant (17.55 and 18.44), 100-seed weight (74.87 and 76.11 g), seed yield/plant (43.692 and 45.244 g) and seed yield/fed (1.481 and 1.529 tons) in the two seasons of the study, respectively.

The superiority of Giza 716 in yield and yield component were found in many previous studies as Kandil *et al*, (2011) whom obtained the highest seed yield per plant and feddan from Giza 716 while Giza 40 and Giza 843. Also, El-Karamity *et al*, (2017) showed that both Giza 716 and Misr 1 recorded more yield than Nubaria 1, Sakha 1 and Giza 843 in both seasons of their study. The variations among faba bean cultivars in yield traits almost due the wide genetic variation among these genotypes (Abido and Seadh, 2014 and Tarek *et al.*, 2020).

1.3. Seed quality traits

Seed contents of both total protein and carbohydrate differ significantly in all tested faba bean genotypes in both seasons in Table (5). Nubaria 1 had the highest seed contents of total protein (44.09 and 44.17%) and total carbohydrates (26.77 and 26.82 %) in both seasons, followed by Nubaria 5 and Giza 716 in the two seasons. On the other hand, Sakha 4 had the lowest total protein (36.02 and 35.55%) while Giza 843 recorded the lowest seed content of total carbohydrates (19.76 and 20.23 %) in both seasons, respectively.

Similar results were found before by Ibrahim, (2016) who found significant differences among faba bean genotypes in all seed quality traits and El-Karamity *et al.* (2017) whom found a wide diversity among faba bean genotypes in seed quality where, Giza 716 had more seed content of total protein than Nubaria 1, Sakha 1 and Giza 843.

2. SCoT analysis

The analysis of seven SCoT primers applied to the assessed Faba bean genotypes revealed noteworthy results (Table 6 and Fig. 1). Each primer generated a total of polymorphic bands, with no monomorphic bands detected in any of the samples. Specifically, SCoT 1 yielded 6 polymorphic bands, resulting in a total of 6 bands and achieving 100% polymorphism. Similarly, SCoT 2 produced 5 unique and 2 polymorphic bands, leading to a total of 7 bands, also achieving 100% polymorphism. SCoT 3 resulted in 3 polymorphic bands, which included 2 unique bands, bringing the total to 5 bands while maintaining 100% polymorphism. SCoT 4 generated 3 polymorphic bands, accompanied by 1 unique band, totaling 4 bands, thus achieving 100% polymorphism as well.

In addition, SCoT 5 produced 2 unique and 4 polymorphic bands for a total of 6 bands, also reaching 100% polymorphism. SCoT 13 yielded 4 polymorphic bands, resulting in a total of 4 bands and maintaining 100% polymorphism. Lastly, SCoT 22 generated 3 polymorphic and 1 unique bands, leading to a total of 4

bands, and achieved 100% polymorphism. Overall, the analysis demonstrates that all SCoT primers utilized in this study exhibited high levels of polymorphism, underscoring their effectiveness in identifying genetic variation among the assessed Faba bean cultivars. The analysis of seven SCoT markers across seven faba bean cultivars revealed distinct patterns of genetic diversity (Fig. 2).

The cultivars clustered into groups based on marker similarity, indicating varying degrees of genetic relatedness. Giza 716 and Giza 843 showed moderate diversity, sharing some marker expression patterns, while Nubaria 2 and Nubaria 5 exhibited unique profiles with distinct marker intensities. Sakha 4 displayed a moderate level of similarity with other cultivars but retained certain unique marker expressions. Wady 1 stood out as the most genetically distinct cultivar, characterized by predominantly low marker expression levels. The intensity of colors in the heatmap, ranging from red (high expression) to blue (low expression), highlighted the activity of specific markers across the cultivars. This high polymorphism level underscores the potential of SCoT markers in detecting genetic diversity within and among faba bean cultivars. Each primer generated polymorphic bands exclusively, with no monomorphic bands detected, highlighting their ability to discern genetic differences effectively.

Comparing these findings with prior studies, the results align with Nosair (2016), who observed 93.99% polymorphism across Leguminosae species using SCoT markers. Similarly, Essa *et al.* (2023) reported a high level of polymorphism (70.93%) across eight faba bean cultivars using six SCoT primers. These consistent findings across different studies reinforce the robustness of SCoT markers in identifying genetic variation within leguminous crops. This assertion is supported by Albrifcany and Askander (2022), who highlighted the reliability of the SCoT technique in evaluating genetic diversity among faba bean cultivars. Moreover, the discrimination power of these markers, as discussed by Tessier *et al*. (1999), indicates their efficiency in distinguishing closely related genotypes. This feature is particularly beneficial in breeding programs, where genetic similarity and distance assessments facilitate the selection of desirable genotypes from segregating or backcrossing populations (Kumawat *et al.,* 2020). The clustering patterns observed in this study may reflect genetic relationships among the assessed faba bean cultivars. In conclusion, the present study confirms the reliability and efficiency of SCoT markers in assessing genetic diversity among faba bean cultivars. These markers demonstrate a strong potential for identifying and discriminating lines. Their application in breeding programs and genetic diversity studies could facilitate the development of improved faba bean cultivars with desirable agronomic traits.

The PCA plot (Fig. 3) illustrates the genetic diversity among seven faba

bean cultivars based on data obtained from seven SCoT molecular markers. The two principal components (PC1 and PC2) explain 45.2% and 21.4% of the total genetic variation, respectively. The cultivars are distributed across the plot, reflecting their genetic relationships. Nubaria 2, Nubaria 5, Giza 716, and Giza 843 are grouped closer together, indicating higher genetic similarity among these cultivars. Sakha 4 shows moderate separation, suggesting a certain level of genetic divergence. Wady 1 is distinctly separated from the other cultivars, highlighting its unique genetic profile. This distribution emphasizes the effectiveness of SCoT markers in distinguishing genetic variation among faba bean cultivars, providing valuable information for genetic improvement and breeding strategies.

3. ITS analysis

Molecular characterization is an important step to identify different plant species. Internal Transcribed Spacer (ITS) regions between genes have been suggested as useful for inferring phylogenetic relationships among closely related organisms (Gurtler and Stanisich, 1996). PCR using genomic DNA of faba bean genotypes as template and ITS primers, produced one fragment of ~750 bp (Fig. 4).

The sequence identity matrix for the seven faba bean cultivars offers a comprehensive comparison of genetic similarities among them. The values represent the percentage of sequence identity between pairs of cultivars (Table 7). For example, the comparison between cultivars Nubaria 1 and Nubaria 2 shows a sequence identity of 22.5%, while the identity between cultivars Nubaria 1 and Nubaria 5 is 24.6%. When comparing cultivar Nubaria 1 to cultivar Sakha 4, the sequence identity is 26.0%. The highest similarity for cultivar Nubaria 1 is with cultivar Giza 843, which has a sequence identity of 21.8%. For cultivar Nubaria 2, there is a 26.2% sequence identity with cultivar Nubaria 5 and 27.3% with cultivar Giza 843, indicating a closer genetic relationship with Giza 843 compared to the other cultivars.

Cultivar Nubaria 5 shares a sequence identity of 26.0% with both cultivars Sakha 4 and Giza 843. In contrast, cultivar Giza 716 displays lower identity percentages, ranging from 15.6% to 16.7% in comparisons with other cultivars with the highest identity of 16.6% occurring with cultivar Nubaria 2. Finally, cultivar Wady 1 shows a sequence identity of 24.4% with cultivar Sakha 4 and 26.4% with cultivar Nubaria 5, while its lowest identity is with cultivar Giza 716 at 15.1%. Generally, this sequence identity matrix provides valuable insights into the genetic relationships among the faba bean cultivars, highlighting various degrees of similarity that can be further investigated in terms of genetic diversity and breeding programs.

The dendrogram (Fig. 5) provides a clear visualization of the genetic relationships among the seven faba bean cultivars, constructed using Internal Tran-

scribed Spacer (ITS) sequence analysis. This analysis leverages the ITS regions high variability, a characteristic that makes them particularly useful for resolving phylogenetic relationships among closely related organisms (Ward and Adams, 1998 and Gurtler and Stanisich, 1996). These regions, located between the conserved ribosomal DNA sequences such as 18S, 5.8S, and 28S rDNA genes (Kularatne *et al*., 2004), serve as a robust tool for investigating genetic diversity. The dendrogram's clustering patterns and branch lengths reflect genetic similarities and differences among the cultivars. Shorter branches, as observed for Nubaria 1 and Nubaria 2, suggest close genetic relationships, potentially indicating shared ancestry or similar selection pressures. This clustering aligns with the hypothesis that genetically similar cultivars tend to group together in phylogenetic analyses (Ward, 1994). The high bootstrap support values associated with this cluster further reinforce the reliability of these findings.

In contrast, Sakha 4 occupies a separate branch, indicating moderate genetic divergence from Nubaria 1 and Nubaria 2. This divergence might be attributable to unique genetic traits or breeding history that distinguishes it from the Nubaria group. Similarly, Giza 843 and Wady 1 are distinct not only from each other but also from the other cultivars, showcasing their unique genetic characteristics. The placement of Nubaria 5 and Giza 716 at the far ends of the dendrogram highlights their substantial genetic divergence, which could be due to significant differences in their genetic makeup or evolutionary pathways. This ITS-based phylogenetic analysis demonstrates the utility of ribosomal DNA regions in elucidating genetic relationships.

Previous studies have emphasized that ITS regions are particularly valuable for such analyses due to their variability (Pramateftaki *et al.*, 2000 and Zhao *et al.*, 2024). The insights gained from this study can have practical applications in breeding programs, as genetically diverse cultivars like Nubaria 5 and Giza 716 may serve as valuable resources for introducing novel traits. Additionally, this genetic information can aid in conservation strategies, helping prioritize efforts to maintain the genetic diversity of Faba bean cultivars. In summary, the study underscores the significance of ITS sequences as a phylogenetic tool and highlights their applicability in understanding genetic relationships, as also noted by Ward and Adams (1998). Future studies could expand upon these findings by integrating additional molecular markers or exploring environmental factors that may have influenced the genetic divergence observed among the cultivars.

Conclusion

The field experiment revealed that Giza 716 cultivar was the most yielded genotype while Nubaria 1 recorded the highest seed quality. The present study highlighted the effectiveness of SCoT and ITS molecular markers in uncovering genetic diversity among seven faba bean

cultivars cultivated in Egypt. The analysis showed 100% polymorphism across all SCoT primers, highlighting their ability to detect significant genetic variation. The sequence identity matrix and ITS-based phylogenetic analysis further emphasized varying levels of genetic similarity among the genotypes, with Nubaria 1 and Nubaria 2 forming a close cluster and other genotypes, such as Wady 1 and Sakha 4, exhibiting unique genetic traits. These findings provide a comprehensive understanding of the genetic structure of faba bean cultivars, underscoring the utility of SCoT markers for genotype identification and marker-assisted selection. This research offers valuable insights for enhancing breeding programs, promoting genetic improvement, and conserving genetic resources in faba beans, which are critical for sustaining their cultivation and adapting to agricultural challenges.

SUMMARY

Evaluation of growth, yield and quality parameters as well as, molecular variation among seven Egyptian faba bean (Nubaria 1, 2, 5, Sakha 4, Giza 716, Giza 843 and Wady 1) cultivars using ITS and SCoT molecular markers were conducted in this present investigations. Field experiments confirmed highly significant diversity among all tested faba bean cultivars in all tested parameters during the two tested seasons. The highest emergence percentages were recorded in Giza 843 cultivar. The obtained results showed that while Giza 716 cultivar was superior to all other tested cultivars in almost growth and yield traits during both seasons. Nubaria 1 had the highest values of seed total protein and carbohydrates content in both seasons. Molecular studies with seven SCoT primers showed that all studied cultivars exhibited 100% polymorphism. SCoT 1 and SCoT 2 produced 6 and 5 polymorphic bands, respectively, while SCoT 4 yielded 4 polymorphic bands with 2 unique bands. The study also utilized Principal Component Analysis (PCA), which explained 66.6% of the total genetic variation, showing close clustering of Nubaria 2, Nubaria 5, Giza 716, and Giza 843, while Wady 1 displayed clear genetic divergence. ITS-based phylogenetic analysis clustered Nubaria 1 and Nubaria 2 closely, while Sakha 4, Giza 843, and Wady 1 exhibited distinct genetic traits. Finally, this investigation showed that under both agronomical and molecular levels, there were a clear variation among almost of studied faba bean cultivars, which might be provide good material for breeding programs and genetic improvement of faba beans.

REFERENCES

Abdelaal M. S. M., (2023). Response of Growth, Productivity and Quality For Some Faba Bean (*Vicia faba* L.) Cultivars to Different Irrigation Regimes. Egypt. J. Agron. Vol. 45, No. 3, pp. 231-247.

Abdel-Razzak H. S., Alfrmawy A. M., Ibrahim H. M. and El-Hanafy A. A., (2012). Genetic Diversity in Faba Bean (*Vicia faba* L.) Using Inter-Simple Sequence Repeat

(Issr) Markers and Protein Analysis. Life Sci. J. 2012, 9: 497-503.

Abid G., Mingeot D. and Udupa S. M., (2015). Genetic relationship and diversity analysis of faba bean (*Vicia Faba* L. var. Minor) genetic resources using morphological and microsatellite molecular markers. Plant molecular biology reporter, 33(6):1755-1767.

Abido W. A. E. and Seadh S. E., (2014). Rate of variations between field bean cultivars due to sowing dates and foliar sparing treatments. Sci. International, 2 (1):1-12.

Abo-Hegazy S. R. E. and Darwish D. S. (2022). Genotype x Environment Analysis of Egyptian Faba Bean Cultivars and their Resilience to Different Sowing Dates in Egypt. Scientific Journal of Agricultural Sciences, 4 (1): 102-113.

Albrifcany M. and Askander H. S. A., (2022). Genetic Diversity Among some Parental Varieties of *Vicia faba* L. Using ISSR and SCoT Molecular Marker Techniques. J. Agric. Chem. Biotechnol., 13, 129-137.

AOAC (1988). Official Methods of Analysis 21st Ed., Association of Official Agricultural Chemists, Washington, D. C., USA.

Aswathy L., Jisha R. S. and Masand V. H., (2017). Computational strategies to antimalarial thiazine alkaloid lead compounds based on an Australian marine sponge *Plakortislita*. J Biomol Struct Dyn., 35(11): 2407-2429.

Bakry B. A., Elewa T. A., El Karamany M. F., Zeidan M. S. and Tawfik M. M., (2011). Effect of row spacing on yield and its components of some faba bean varieties under newly reclaimed sandy soil condition. World J. Agric. Sci., 7(1):68-72.

Bhattacharyya P., Kumaria S., Kumar S. and Tandon P., (2013). Start Codon Targeted (SCoT) Marker Reveals Genetic Diversity of Dendrobium Nobile Lindl., an Endangered Medicinal Orchid Species. Gene, 529: 21-26.

Collard B.C. Y. and Mackill D. J., (2009). Start codon targeted (SCoT) polymorphism: a simple, novel DNA marker technique for generating gene-targeted markers in plants. Plant Mol. Biol. Rep., 27(1):86-93.

Doyle J. J. and Doyle J. L., (1990). Isolation of plant DNA from fresh tissue. Focus 12: 149-151.

Dubois M., K. A. Gilles, Hamilton J. R. and Smith F., (1956). Colorimetric method for determination of sugar and related substance. Annal. Chem., 28: 350-356.

Duc G., Bao S., Baum M., Redden B., Sadiki M., Suso M. J., Vishniakova M. and Zong X., (2010). Diversity Maintenance and Use of *Vicia faba* L. Genetic Resources. Field Crop. Res., 115: 270-278.

El-Karamity A. E., Abdulla S. Sh. and El-Ridwany A. R., (2017). Effect of plant distribution and density on yield and yield components of some faba bean (*Vicia faba* L.) cultivars. Minia J. of Agric. Res. & Develop. 37(2): 211-230.

Ellwood S. R., Phan H. T. T., Jordan M., Hane J., Torres A. M., Avila C. M., Cruz-Izquierdo S. and Oliver R. P., (2008). Construction of a Comparative Genetic Map in Faba Bean (*Vicia faba* L.); Conservation of Genome Structure with Lens Culinaris. BMC Genom., 9, 380.

Essa S. M., Wafa H. A., Mahgoub E.-S. I., Hassanin A. A., Al-Khayri J. M., Jalal A. S., El-Moneim D. A., Alshamrani S. M., Safhi F. A. and Eldomiaty A. S., (2023). Assessment of Eight Faba Bean (*Vicia faba* L.) Cultivars for Drought Stress Tolerance through Molecular, Morphological, and Physiochemical Parameters. Sustainability.;15:3291.

FAOSTAT. (2021). Production Statistics of the Food Agriculture Organization of the United States. Availabl online:http://www.fao.org/faostat/en/#data/QA (accessed on 3 June (2021).

GelAnalyzer, Version three, (2007). Gel Analyzer Ver.3 program software for windows. www.geocities. Com /egygene.

Gomez K. A. and Gomez A. A., (1984). Statistical procedures for agricultural research (2 ed.). John wiley and sons, New York, 680p.

Gorji A. M., Poczai P., Polgar Z. and Taller J., (2011). Efficiency of Arbitrarily Amplified Dominant Markers (SCoT, ISSR and RAPD) for Diagnostic Fingerprinting in Tetraploid Potato. Am. J. Pot. Res., 88:226-237.

Gupta D. D., Hui P. K. and Tag H., (2018). Genotypic variation in Acmella paniculata across different phytogeographical ranges of Northeast India inferred through ISSR & SCoT based markers. J. Appl. Res. Med. Aromat. Plants, 11:3-11.

Gürtler V. and Stanisich V. A., (1996). New approaches to typing and identification of bacteria using the 16S-23S rDNA spacer region. Microbiology, 142: 3-16.

Ibrahim H. M., (2016). Performance of some faba bean (*Vicia faba* L.) cultivars sown at different dates. Alexandria Science Exchange Journal, 37(2): 175-186.

Kandil A. A., Sharief A. E. M. and Mahmoud A. S. A., (2011). Reduction of flower dropping in some faba bean cultivars by growth regulators foliar application. J. Plant Production, Mansoura Univ., Vol. 2 (11):1439- 1449.

Kularatne H. A. G. C., Lawrie A. C. and Barber P. A., Keane P. J, (2004). A specific primer PCR and RFLP assay for the rapid detection and differentiation in planta of some Mycosphaerella species associated with foliar diseases of Eucalyptus globulus. Mycological Research, 108: 1476-1493.

Kumawat G., Kumawat C.K., Chandra K., Pandey S. P., Chand S., Mishra U. N., Lenka D. and Sharma R., (2020). Insights into Marker Assisted Selection and Its Applications in Plant Breeding. In Plant Breeding Current and Future Views; IntechOpen: London,UK,. 63.

Maalouf F., Hu J., O'Sullivan D. M., Zong X., Hamwieh A., Kumar S. and Baum M., (2018). Breeding and genomics status in faba bean(*Vicia faba*). Plant Breed., 138: 465-473.

Nosair H., (2016). SCoT polymorphism reveals genetic diversity in some important Fabaceae species. Curr. Sci. Int., 5: 592-598.

Pramateftaki P. V., Lanaridis P. and Typas M. A., (2000). Molecular identification of wine yeasts at species or strain level: a case study with strains from two vine-growing areas of Greece. J. Appl. Microbiol., 89, 236-248.

Sambrook J., Fritsch E. F. and Maniatis, T., (1989). Molecular cloning: A Laboratory Manual Cold Spring Harbor, New York: Cold Spring Harbor Lboratory Press.

Soliman A. A., Ibrahim M. A., Mousa M. I., Mansour E., He Y. and Yu H., (2024). Genetic potential and inheritance pattern of agronomic traits in faba bean under free and infested Orobanche soil conditions. BMC Plant Biology 24:301.

Tarek S. M., Mohamed Marwa Kh. A. and Rehab A. M. A., (2020). Evaluation of some faba bean genotypes under three planting dates in middle Egypt. Alex. J. Agric. Sci., 65(3):201-209.

Tawfik M. M., Bakhoum Gehan Sh., Kabesh M. O. and Thalooth Alice T., (2018). Comparative study on some faba bean cultivars under water limitation conditions and different sowing dates. Middle East Journal of Agriculture Research., 07(04):1431-1443.

Tessier C., David J., This P., Boursiquot J. M. and Charrier A., (1999). Optimization of the choice of molecu-

lar markers for varietal identification in *Vitis vinifera* L. Theor. Appl. Genet., 98: 171-177.

Ward E. and Adams M. J., (1998). Analysis of ribosomal DNA sequences of *Polymyxa* species and related fungi and the development of genus- and species-specific PCR primers. Mycological Research 102: 965-974.

Ward E., (1994). Use of the polymerase chain reaction for identifying plant pathogens. In Ecology of Plant Pathogens (ed. J. P. Blakeman & B. Williamson), pp. 143-160. C. A.B. I . Wallingford, U.K.

Willett W., Rockström J., Loken B., Springmann M., Lang T., Vermeulen S., Garnett T., Tilman D., DeClerck F., Wood A., et al. (2019). Food in the Anthropocene: The EAT-Lancet Commission on healthy diets from sustainable food systems. Lancet, 393, 447-492.

Zhao Zy., Wu Jw. and Xu Cg. *et al.* (2024). Molecular identification and studies on genetic diversity and structure-related GC heterogeneity of *Spatholobus Suberectus* based on ITS2. *Sci Rep.* 14, 23523.

Zong X., Liu X., Guan J., Wang S., Liu Q., Je V. and Redden R., (2009). Molecular variation among Chinese and global winter faba bean germplasm. Theor Appl Genet 118(5):971- 978.

Table (1): Names, pedigree and country of origin of the seven Egyptian faba bean cultivars used in this study.

Cultivars	Pedigree	Country of origin
Nubaria 1	Selection in Rena Blanka	Egypt
Nubaria 2	ILB1550 x Radiation 2095/76	Egypt
Nubaria 5	landraces of Hamam 10	Egypt
Sakha 4	Sakha 1 x Giza 3	Egypt
Giza 716	461/842/83/503/453/83	Egypt
Giza 843	561/2076/85 Sakha x461/845/83	Egypt
Wady 1	Rena Blanka x Triple white	Egypt

Table (2): Base sequences of used seven SCoT primers.

No.	**Primers**	**Sequence**
1	SCoT 1	5'-CAACAATGGCTACCACCA-3'
2	SCoT 2	5'-CAACAATGGCTACCACCC-3'
3	SCoT 3	5'-CAACAATGGCTACCACCG-3'
4	SCoT 4	5'-CAACAATGGCTACCACCT-3'
5	SCoT 5	5'-CAACAATGGCTACCACGA-3'
6	SCoT 13	5'-ACGACATGGCGACCATCG-3'
7	SCoT 22	5'-AACCATGGCTACCACCAC-3'

Table (3): Growth traits of the tested faba bean cultivars during 2023 and 2024 seasons.

Cultivars	**Emergency (%)**		**Maturity date (days)**		**Plant height (cm)**		**Number of branches**	
	1st S	**2nd S**	**1st S**	**2nd S**	**1st S**	**2nd S**	**1st S**	**2nd S**
Nubaria 1	87.14	89.21	154.46	150.67	87.35	89.07	3.64	4.06
Nubaria 2	87.93	87.46	146.19	145.82	94.60	93.23	2.78	3.14
Nubaria 5	88.40	87.28	143.90	145.34	91.85	90.49	4.11	4.05
Sakha 4	95.60	95.34	140.58	141.67	81.17	83.86	3.27	2.98
Giza 716	87.38	88.06	136.80	134.28	110.26	115.71	4.58	5.02
Giza 843	96.81	96.52	144.00	145.60	79.92	81.52	2.66	2.74
Wady 1	94.99	96.17	150.34	148.7	83.54	85.06	3.12	3.51
LSD 5%	**1.46**	**1.45**	**1.96**	**1.78**	**3.50**	**3.84**	**0.23**	**0.26**

Table (4): Yield traits of the test faba bean cultivars during 2023 and 2024 seasons.

Cultivars	Number of pods/plant		100-seed weight (g)		Seed yield/plant (g)		Seed yield/feddan (tons)	
	1st S	2nd S	1st S	2nd S	1st S	2nd S	1st S	2nd S
Nubaria 1	20.24	20.87	103.21	102.82	57.397	58.078	1.751	1.814
Nubaria 2	21.11	21.69	97.36	96.14	50.789	52.384	1.563	1.604
Nubaria 5	22.42	23.04	100.64	102.2	59.002	60.694	1.826	1.854
Sakha 4	18.65	19.21	82.19	80.33	44.560	46.524	1.491	1.553
Giza 716	24.79	24.67	92.74	94.09	63.175	64.099	1.932	1.976
Giza 843	17.55	18.44	74.89	76.11	43.692	45.244	1.481	1.529
Wady 1	19.54	19.71	78.35	79.65	45.480	48.440	1.512	1.631
LSD 5%	**0.81**	**0.74**	**3.79**	**3.75**	**2.62**	**2.47**	**0.06**	**0.06**

Table (5): Seed quality traits of the seven test faba bean cultivars during 2023 and 2024 seasons.

Cultivars	Total proteins (%)		Total Carbohydrates (%)	
	1st S	2nd S	1st S	2nd S
Nubaria 1	44.09	44.17	26.77	26.82
Nubaria 2	42.31	42.08	24.69	24.75
Nubaria 5	43.95	44.73	26.61	27.15
Sakha 4	36.02	35.55	21.12	21.14
Giza 716	41.97	42.42	25.99	26.36
Giza 843	38.52	41.11	19.76	20.23
Wady 1	40.79	39.74	20.64	21.35
LSD 5%	**0.98**	**1.03**	**1.01**	**0.99**

Table (6): Number of polymorphic, monomorphic and unique PCR bands and percentage of polymorphism obtained utilizing seven SCoT primers for the assessed faba bean cultivars.

Primers	Monomorphic bands	Unique bands	Polymorphic bands		Total number of bands	Polymorphism (%)
			without Unique	with Unique		
SCoT 1	0	0	6	6	6	100
SCoT 2	0	5	2	7	7	100
SCoT 3	0	2	3	5	5	100
SCoT 4	0	1	3	4	4	100
SCoT 5	0	2	4	6	6	100
SCoT 13	0	0	4	4	4	100
SCoT 22	0	1	3	4	4	100

Table (7): Sequence identity matrix for the seven faba bean cultivars.

Sequence Identity Matrix	Nubaria 1	Nubaria 2	Nubaria 5	Sakha 4	Giza 716	Giza 843	Wady 1
Nubaria 1	-						
Nubaria 2	0.225	-					
Nubaria 5	0.246	0.262	-				
Sakha 4	0.260	0.234	0.260	-			
Giza 716	0.167	0.166	0.157	0.156	-		
Giza 843	0.218	0.273	0.258	0.276	0.156	-	
Wady 1	0.200	0.224	0.264	0.243	0.151	0.236	-

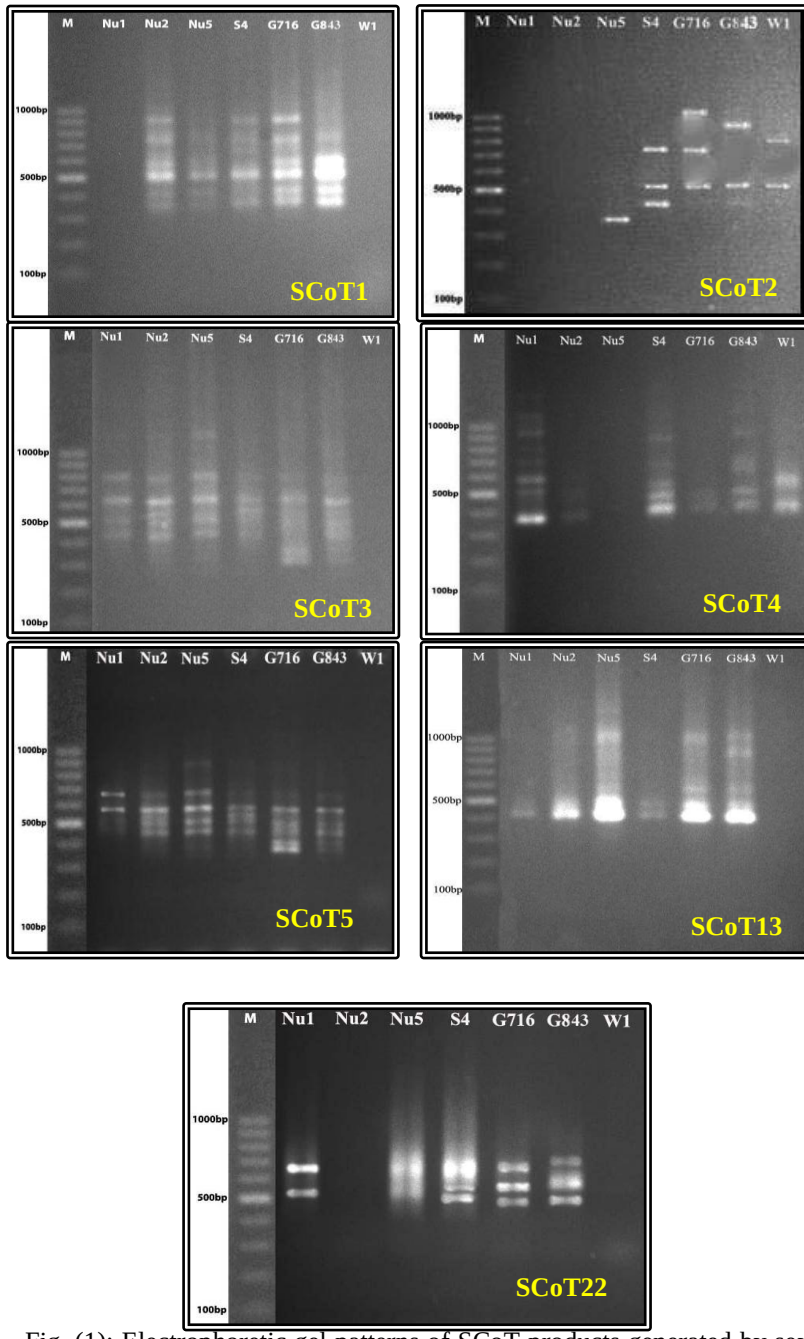

Fig. (1): Electrophoretic gel patterns of SCoT products generated by seven SCoT primers (SCoT1, SCoT2, SCoT3, SCoT4, SCoT5, SCoT6 and SCoT 7).

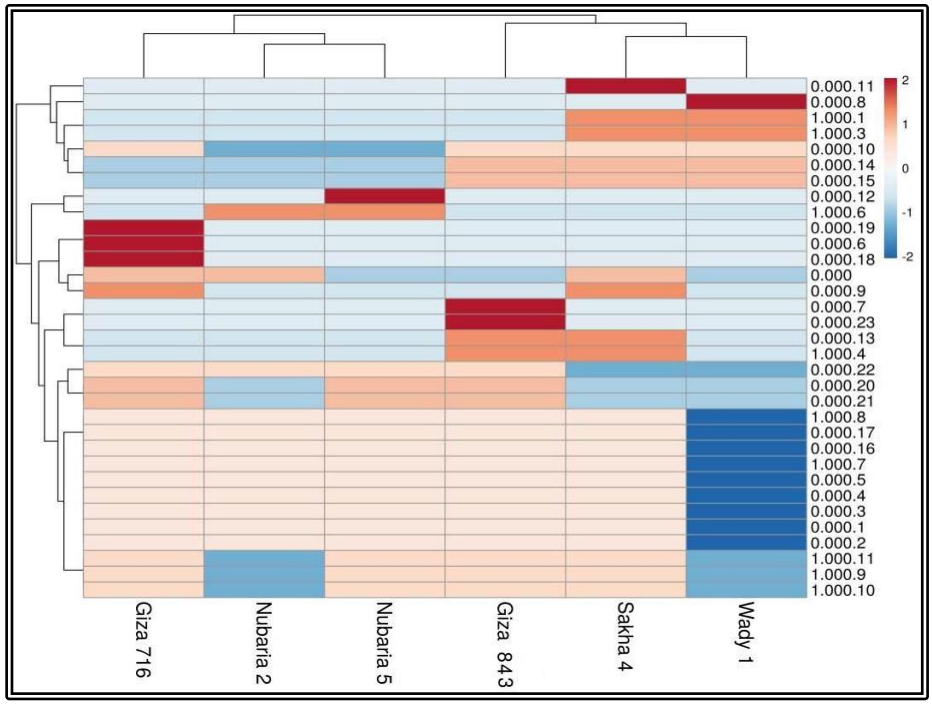

Fig. (2): Multivariate heatmap illustrating the genetic diversity of the tested seven faba bean based on the seven SCoT primers using the module of heatmap of https://biit.cs. ut.ee/clustvis/software.

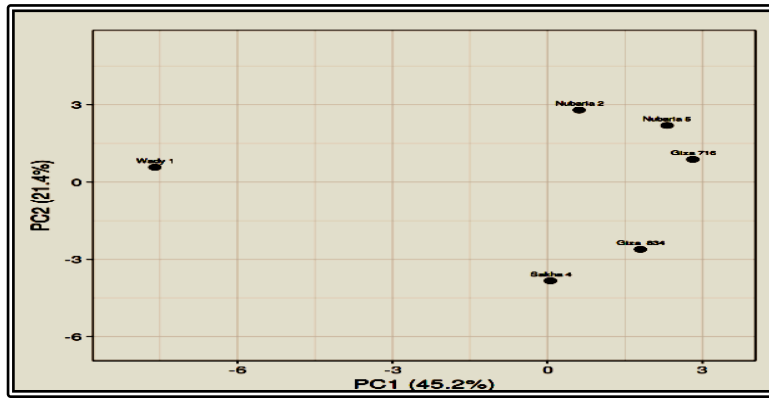

Fig. (3): Principle component analysis (PCA) scatter diagram illustrating the genetic diversity expressed by the grouping of seven species of bean based on the analysis of seven SCoT marker polymorphism using PAST software.

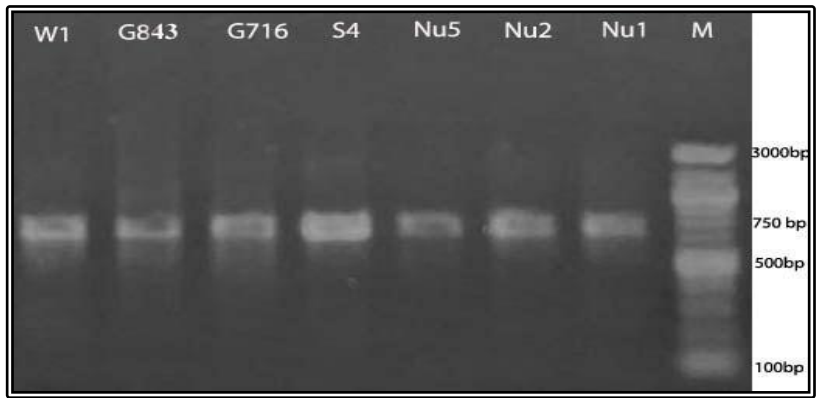

Fig. (4): ITS gene electrophoretic patterns of some Egyptian faba bean cultivars, M: 100 bp Ladder marker.

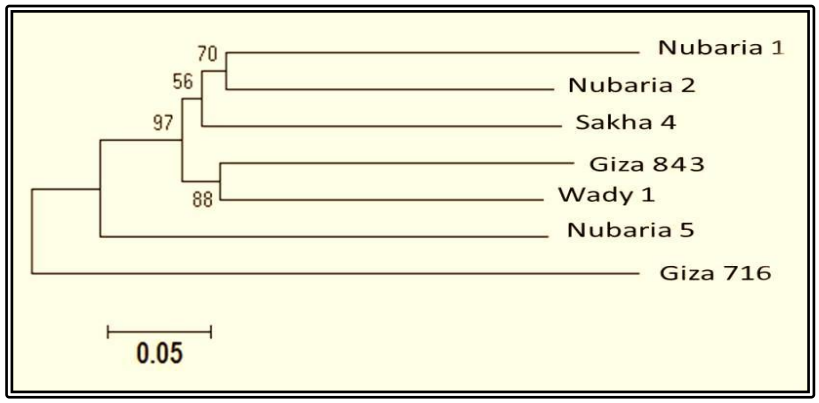

Fig. (5): Dendrogram of the genetic distances among studied faba bean cultivars based on ITS region.