



Genetic Variability and ISSR Markers of some Faba bean (*Vicia faba* L.) cultivars under drought condition

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ABSTRACT

This work was carried out for genetic differentiation for drought tolerance of five Egyptian faba bean cultivars (Sakha 1, Sakha-4, Nubaria-1, Giza-716, and Giza-843) were planted under three levels of field capacity (FC) irrigation doses; 100%, 75% and 50% FC respectively with split-plot design in two winter seasons in 2019/2020 and 2020/2021. Growth, yield and its attributes characters, chlorophyll content, stress tolerance indices, and genetic variation were recorded. Five primers of Inter-Simple Sequence Repeat (ISSR) molecular markers were used to detect the variability. Drought caused a significant reduction in growth, yield, and its attributes characters and chlorophyll. Giza843 has a high yield, and its attributes characters under all watering treatments. At the same time, Sakha1 and Sakha4 showed increased sensitivity to drought conditions, although Sakha4 gave high yield and its component under normal conditions while Giza716 cultivar showed high yield under severe conditions. The phenotypic coefficient of variation (PCV) value was higher than the genotypic coefficient of variation (GCV) value for all studied traits. One hundred seeds weight trait showed the highest PCV (71.15%), GCV (70.86%), and Genetic Advance (GA) (145.38), while the root length trait showed the lowest PCV (6.49%), GCV (6.04%), and GA (11.59). Higher values of heritability and GA were obtained for pods and leaves number/ plant, pods weight / plant, and 100 seeds weight. Results of tolerance indices indicated that Giza 716 and Giza843 cultivars (cv.) were drought-tolerant cultivars while other studied cultivars were sensitive. ISSR analysis identified 37 loci and heterozygosity ranged from 0.736 to 0.892 with an average of 0.827. Fourteen bands were polymorphic, with 37.83% percent of polymorphism. Polymorphism information content (PIC) values were ranged from 0.75 to 0.89, averaging 0.84. Ten unique bands were used as molecular markers, most obtained by HB12 primer. ISSR markers' cluster analysis divided five cultivars into three main clusters. Genetic similarity and dendrogram were in harmony with yield traits results.

Keywords: Water stress, Genetic Variation, ISSR-PCR markers, Polymorphic information content (PIC), and Tolerance Indices.

1. Introduction

Faba bean (*Vicia faba* L.) is the essential food legume in Egypt, which consumed in the succulent green state and dry edible seeds commonly regarded as a major protein source due to high seed content of crude protein and critical amino acid (Vogelsang-O'Dwyer *et al.*, 2020). Faba bean is a diploid legume ($2n = 2x = 12$), contain size genome ~13,000 Mb and partly allogamous (4-84%) (Hamadache and Grandes 2014). nodules of faba bean roots increase the fertility of marginal lands by

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fixing atmospheric N consequently increase the ability of growing and yield effectively (Farag and Afiah 2012). Biotic and abiotic stress led to had decline effect in faba bean productivity, with the lowest yield growth rate among legumes (Alghamdi 2007 and Maalouf *et al.*, 2019). In arid and semiarid regions; drought is a spacious spread problem that seriously influences crop production which effect on all features of plant growth (Golbashi *et al.*, 2010).

The genetic development of faba bean for drought resistance by traditional methods of breeding is time-consuming and difficult because of the effect of selection and adaption at several locations (Abdul Muktadir *et al.*, 2020). The production of high-yield or tolerant crops is contingent upon the availability of plant genetic resources that may be employed by various breeding programs to generate plants with better features (Yadav *et al.*, 2017). The information on genetic variation and association between agronomic characters supports considerable help to continue genetic improvement in breeding programs. High GCV, PCV, heritability, and GAM values for traits suggest effective indirect selection for grain yield (Jagadev *et al.*, 2021). Most of the characteristics showed high levels of heritability, indicating that variations were transmitted to offspring, suggesting that a high-yielding variety may be created by selecting appropriate genotypes (Magar *et al.*, 2021). Tolerance indices such as drought susceptibility index, geometric mean productivity mean productivity and yield stability index; (DSI), (GMP), (MP) and (YSI) respectively showed high positive correlation with seed yield under stress and normal conditions which are suitable to identify tolerance cultivars or genotype with high yield in drought stress (Mohammadijoo *et al.*, 2015, Mehraban *et al.* 2018, Hooshmandi 2019 and Abd El-Kreem *et al.*, 2019). Drought tolerance was polygenic and complex trait effected with the environment causing difficulty in phenotypic evaluation while; DNA markers using could be help breeders in improving the speed and reliability of the evaluation process (Khan *et al.*, 2013). ISSR technique was much suitable and precisely for resolving genetic diversity of closely related faba bean genotypes and for cultivar identification through high percent of polymorphism was obtained by ISSR primers (El-Nahas *et al.*, 2011, Kianoosh *et al.*, 2017, Asfaw *et al.*, 2018 and Qahtan *et al.*, 2021). specific markers; negative and positive molecular size (pb) for different ISSR primers used as marker assisted selection (MAS) and appear that ISSR markers are effective in faba bean breeding programs and encourage targeted crossing strategies for drought tolerance and genetic relationships between different faba bean cultivars (Belal *et al.*, 2009, Afiah *et al.*, 2016 and Qahtan *et al.*, 2021).

The present study aimed to: evaluate the variability of morphological, yield and molecular traits in five Egyptian faba bean cultivars for improving tolerance to drought stress during growth season.

2. Materials and Methods

The research was established throughout the 2019-2020 and 2010-2021 growing seasons at the experimental farm of the Faculty of Agriculture at Suez Canal University in Ismailia, Egypt. Faba bean (*V. faba* L.) cultivars namely, Sakha-1, Sakha-4, Nubaria-1, Giza-716, and Giza 843 were sown under three field capacity of water treatments; 100% , 75%, and 50% FC until harvest stage. All other agricultural practices were followed as recommended. A split-plot design was used in a randomized complete block arrangement (RCB) with three replications. The main plots were devoted to water treatments. Sub-plots were devoted to five faba bean genotypes. Each experimental plot had three ridges measuring 3 m in length and 60 cm in breadth. On one side of the ridges, each hill was 20 cm apart. Plant samples were taken at harvest stage; ten plants were taken randomly from each plot to measure: Plant height (cm), root length (cm), leaves number /plant, branches number/plant, shoot fresh weight/plant (g), shoot dry weight/plant (g), root fresh weight/plant (g), root dry weight/plant (g), pods number / plant, pods weight / plant (g), seed yield / plant (g) and 100-seed weight (g) traits.

Chlorophyll SPAD values using SPAD-502 chlorophyll-meter (Minolta Co., Ltd., Japan) were determined in plant leaves at the flowering stage three times, and the average was calculated by Meloni *et al.*, (2003) and Yadava *et al.*, (2017).

2.1. Genetic variation

The genotypic variance $\sigma^2 g = M_1 - M_2 / r$

The phenotypic variance $\sigma^2 p = \sigma^2 g + \sigma^2 e$

σ^2_p denotes phenotypic variance, σ^2_g denotes genotypic variance and σ^2_e denotes environmental variance.

M_1 denotes to expect mean squares of genotypes calculated from the ANOVA table. M_2 denotes to expect mean squares of error calculated from the ANOVA table.

The genotypic and phenotypic coefficients of variability; (G.C.V %) and (P.C.V %) respectively were calculated as σ_g/x and σ_p/x , respectively.

2.2. Heritability in a broad sense (h^2) and coefficient of variation (CV) were calculated according to Hallauer and Miranda (1988) for studied traits.

The genetic advance (GA): calculated according to Allard and Bradshaw (1964), was estimated from the following formula: $GA = I h^2 V_p$. where $I = 1.76$ (A 10 % selection intensity), h^2 : heritability in broad sense.

Deficit tolerance and susceptibility indices, including geometric mean productivity (GMP), drought susceptibility index (DSI), mean productivity (MP), yield stability index (YSI), and tolerance (TOL) for water stress environment, were calculated based on seed yield under 50% FC and unstressed (100% FC). The following formulae calculated stress tolerance attributes: (GMP), (DSI),(YSI), (MP) and Tolerance (TOL) were calculated according to Gupta *et al.* (2001) and Chaudhuri and Kanemasu (1982).

1. Tolerance index (TOL) = $Y_p - Y_s$
2. Geometric Mean Productivity (GMP) = $\sqrt{Y_p * Y_s}$
3. Mean productivity = $(Y_p + Y_s)/2$
4. Yield stability index (Y SI) = Y / Y
5. Drought susceptibility index = $(1 - Y_s/Y_p) / DII$

Where : Y_s = mean seeds yield of each genotype under 50% FC condition; Y_p = mean seeds yield of each genotype under 100% FC condition; DII = Drought intensity index.for each water regime (FC%) was calculated as $DII = 1 - X_s/X_p$,

2.3. ISSR-PCR analysis

In the central Lab, Department of Agricultural Botany, Faculty of Agriculture, Suez Canal University, Egypt, genomic DNA of faba bean was extracted using the DNeasy Plant Mini Kit (Qiagen, Valencia, CA) from 100 mg young leaf tissues. Genomic DNA quality and quantity were assessed by NanoDrop spectrophotometer ND-1000 for molecular analysis. Five primers for PCR-based technique ISSR were used; the sequences of these primers are given in Table (1). DNA extraction was performed using the protocol of Dellaporta *et al.* (1983). ISSR analysis was conducted in a 50 μ l reaction comprising 0.2 mM (dNTPs), 1.5 mM $MgCl_2$, 5.0 μ l 10 buffer, 0.2 μ l primer (50 mol), 3.0 μ l template DNA (50 μ g/ μ l), 0.3 μ l Taq DNA polymerase (5 U/ μ l), and up to 50 μ l of sterile water. After a 4-minute initial denaturation cycle at 94 ° C., 40 cycles of PCR amplification were planned. Each cycle included a 1-minute denaturation step at 94 ° C., a 1 minute annealing step at 47 ° C, and a 2 minute elongation stage at 72 ° C. In the last cycle, the primer extension segments lasted 7 minutes at 72 ° C. The ISSR products were examined using ethidium-stained 1.2 percent agarose gel electrophoresis in 1 TBE buffer. The run was conducted for one hour at 120 volts in a Biometra (40 20 cm) submarine. Biometra Bio-Doc Analyze 2005 was used to identify and photograph bands using UV-transilluminator. fragment sizes were determined using a 100–3000 bp DNA ladder.2005. The reproducible bands of DNA for each genotype by each primer were scored in a binary matrix with (1) for presence band and (0) for absence band. The percentage of polymorphism was calculated according to Nei (1973). Effective number of alleles (N_e) calculated according to Wright (1951). Genetic similarity: calculated according to Dice (1945). A dendrogram was created according to (Rohlf, 2000) based on values of similarity using NTSYS-pc version 2.11T.

Table 1. List of ISSR primers and their sequences.

Primer Name	Primer Sequence (5' → 3')
14A	CTC TCT CTC TCT CTC TTG
HB-11	GTG TGT GTG TGT TGT CC
HB-12	GTG TGT GTG TGT TGT CC
HB-13	GAG GAG GAG GC
HB-15	GTG GTG GTG GC

3. Results and Discussion

3.1. Mean performance of genotypes

The mean performances for studied traits of faba bean genotypes under three water regimes are given in Figures (1, 2, 3,4). Mean comparisons were performed by Duncan's Multiple Range Test (DMRTs) at a 0.05 level, where different letters indicate significant differences (Steel and Torrie, 1980).

3.1.1. Growth characters

Results showed that Sakha1, Sakha4, and Nobarey-1 cultivars for plant height and root length traits take the same behavior in gradual decreasing as a response to a reduction in field capacity, although Sakha1 cultivar has the longest plants; 102.3, 95.7, and 90.3 cm under 100%, 75% and 50 % of FC, respectively (Figure 1A) but it has the shortest root length. Giza-716 cultivar was appearing a severe decrease in plant height under 75% and 50% FC conditions, although it has the longest root under all FC conditions; 22.3, 20.3, and 17 cm under 100, 75% FC 50 % FC, respectively, followed by Giza843. Might due to drought conditions induced specific genes that control the root system's development and formation, and there were dominant alleles that controlled the length of roots (Rauf *et al.*, 2007 and Zulu and Modi, 2010). Tolerant genotypes for drought conditions had longer roots under drought conditions than regular irrigation to reach storage water (Khatab *et al.*, 2019). So, this feature could be efficiently fused into breeding programs for drought tolerance.

Giza 843 exhibited the highest values for the number of leaves/plant and branches/ plant (97.7 and 8.3, respectively) under 100% field capacity. In comparison, under 75% and 50 %FC, it was appeared high sensitivity to decreasing field capacity, such as other cultivars. Giza 716 cultivar recorded the highest values (42 and 3.7, respectively) under severe water stress (50%FC).

All cultivars showed a great decrease in fresh and dry weight of roots under 75% and 50% Fc, especially Giza 843 and Sakha1 cultivars under 50%FC (Figure 2). Sakha-4 under 50% FC only gave the highest values for fresh and dry weight of shoot and root. Although plants of the Giza-716 cultivar have a low fresh and dry weight of shoot and root under 100% FC it has moderate weight under drought conditions, and the percent decrease is lower than other cultivars. Drought caused significantly decreasing effects on the growth performance of studied genotypes due to inhibited changes in cell metabolisms (Farooq *et al.*, 2012 and Fouda *et al.*, 2022). Results indicated that the Giza-716 cultivar fit to grow under drought conditions

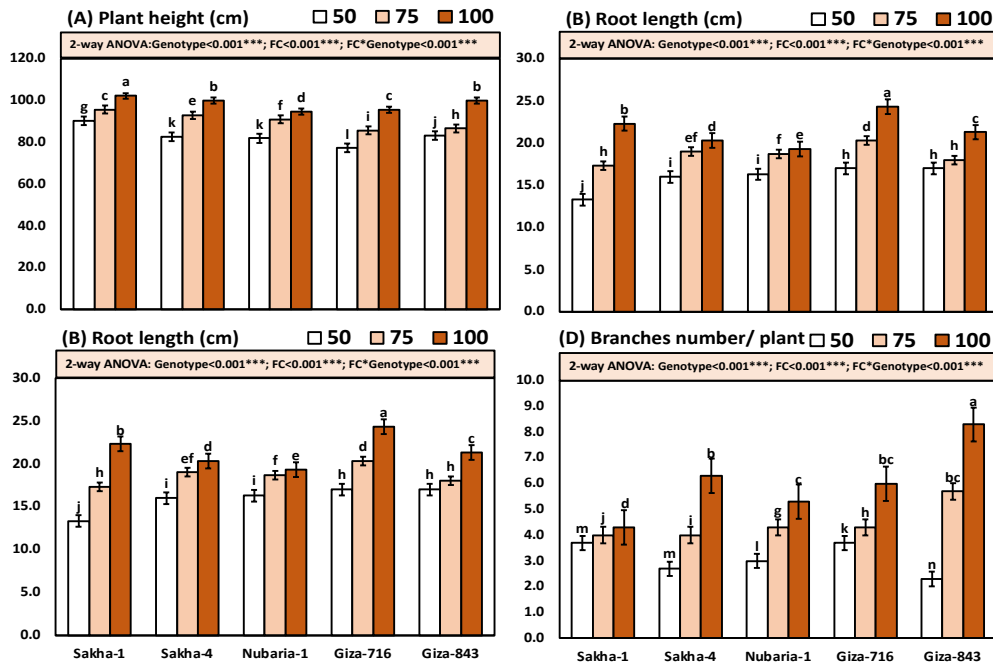


Fig. 1. (A) Shoot fresh weight (g/plant), (B) Shoot dry weight (g/plant), (C) Root fresh weight (g/plant), and (D) Root dry weight (g/plant) in faba bean under drought stress

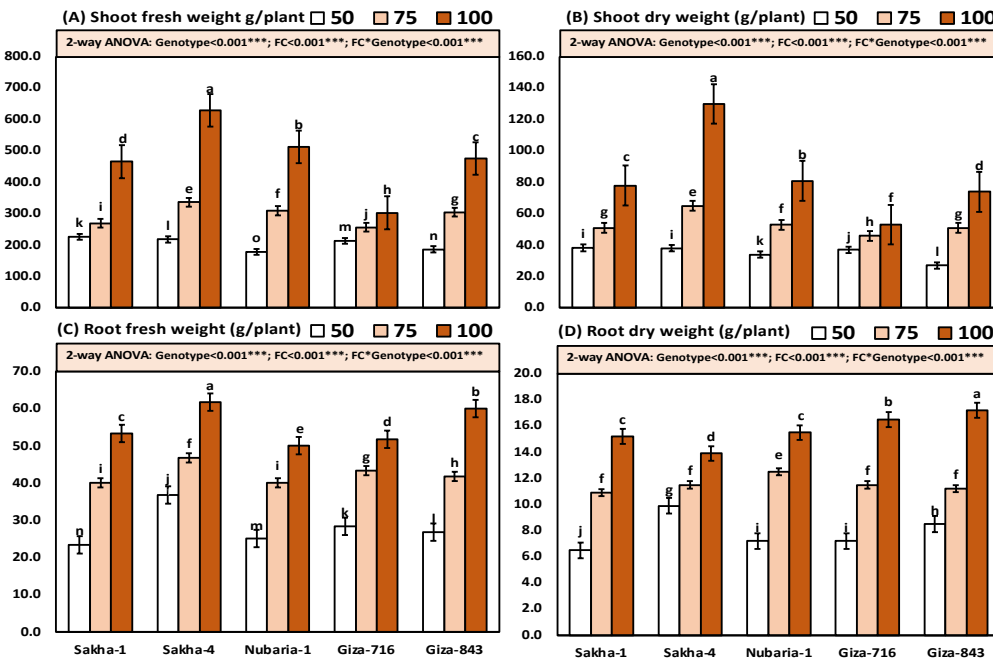


Fig. 2: (A) shoot fresh weight (g/plant), (B) Shoot dry weight (g/plant), (C) Root fresh weight (g/plant), and (D) Root dry weight (g/plant) in faba bean under drought stress.

3.1.2. Yield and its attributes characters

Giza-843 cultivar under three FC% treatments gave the highest mean values for pods weight / plant (g) pods number / plant and seed yield / plant (g). Giza 716 cultivar recorded the highest mean values for the 100-seed weight (g) under all FC%, especially under severe conditions, followed by Giza 843. (Figure 3A-D). While; Sakha 4 cultivar showed the highest mean values for yield traits under 100%FC only

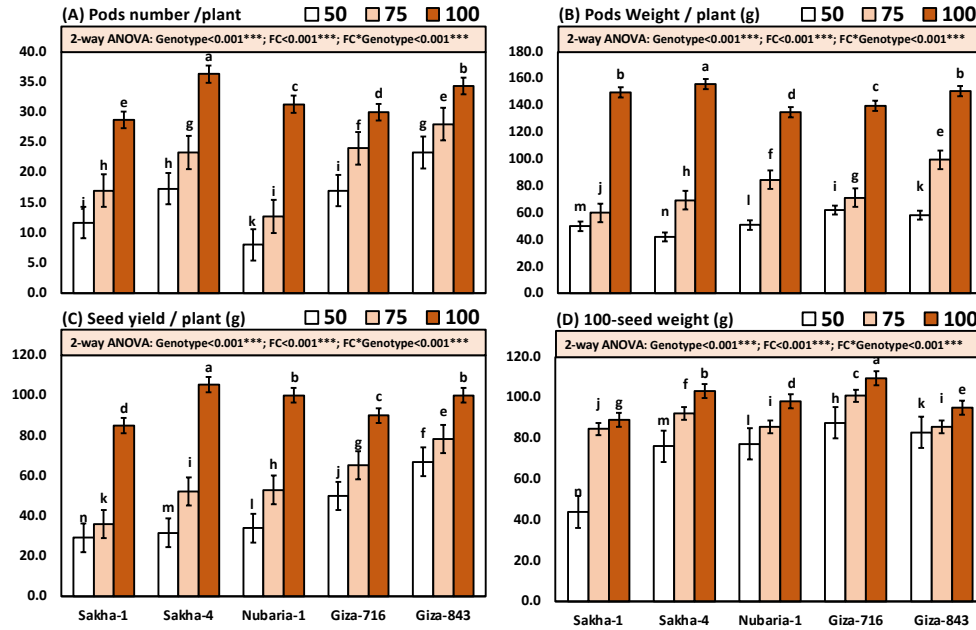


Fig. 3: (A) Pods number/plant, (B) Pods weight (g/plant), (C) seed yield (g/plant), and (D) 100-seed weight (g) in faba bean under drought stress

On the contrary, Sakha 1 exhibited lower values for seed yield/plant (g) and 100-seed weight (g) under three water treatments (Figure 3). Meanwhile, Nobarey-1 cultivars gave the lower values under three water treatments for pods number / plant. A result showed that seed yield per plant (g) was significantly reduced because of the shortage of water treatments from 100% FC to 50 % FC by 59.10%. This reduction was associated with a decrease in all other yield components. Results were in harmony with Khatab *et al.*, (2019), who found that genotypes had highly tolerance for drought conditions decreased the level of damage of yield and its components under stress treatment compared with the control experiment and accepted by. Mansour *et al.*, (2021), Drought-tolerant genotypes provided higher yields with less water than drought-sensitive genotypes and are thus highly recommended for faba bean cultivation in drought situations.

3.1.3. Chlorophyll

Giza 843 cultivar showed the highest Chlorophyll content for all FC% studied; 100% FC (61.7) 75%FC (56) and 50% FC(48.7) followed by Giza716 cultivar compared with other studied cultivars (Figure 4). the imposed to drought stress caused reduction in growth and chlorophyll content by increasing the activity of the chlorophyllase enzyme which causing the degrading of chlorophyll, chloroplast structure, and pigment-protein complexes unstable (Jamil *et al.*, 2012 and Bayoumi *et al.*, 2015). Tolerant cultivars with high chlorophyll content might be due to their ability to repair the damage mechanism, but sensitive cultivars did not have this mechanism (Afiah *et al.*, 2016).

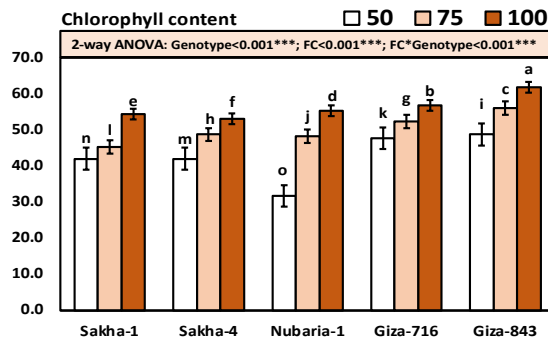


Fig. 4: Chlorophyll contents of faba bean genotypes under drought stress.

3.1.4. Genetic variation

Phenotypic coefficients of variation values ranged from 6.49 for root length trait to 71.15 % for 100 seeds weight (Table 2). The genotypic coefficient of variation also had a similar trend as the phenotypic coefficient of variability from 6.04 to 70.86 for root length and 100 seeds weight traits, respectively. The magnitude of the phenotypic coefficient of variability (PCV) values for all the traits were higher than the corresponding (GCV) values indicating that these traits may be impacted by environmental factors (Mesfin *et al.*, 2019 and Tadele *et al.*, 2021). The broad-sense heritability values were highest for all the studied traits; values ranged from 82.23 to 99.85 for root dry weight and pods weight traits, respectively. The genetic improvement as a percentage of means (GA) for characters ranged from 11.59 for root length trait to 145.38 % for 100 seeds weight trait. The combination among high values of h^2 and GA were found in pods and leaves number/plant, pods weight/ plant, and 100 seeds weight traits offer a clear selection base.

Table 2. Estimation of variance components phenotypic (PCV) (%) and genotypic (GCV) (%) coefficients of variation, broad-sense heritability (h^2) (%) and genetic advance (GA) (%) for each trait.

Traits	PCV (%)	GCV (%)	GA (%)	h^2 (%)
Plant height (cm)	6.98	6.94	14.23	98.98
Root length (cm)	6.49	6.04	11.59	86.65
Leave number /plant	49.48	49.28	101.09	99.17
branches number/plant	21.52	20.11	38.72	87.37
Shoot fresh weight/plant (g)	25.90	25.69	52.49	98.38
Shoot dry weight/plant (g)	6.54	6.50	13.32	98.89
Root fresh weight/plant (g)	12.19	11.71	23.17	92.26
Root dry weight/plant (g)	7.82	7.09	13.24	82.23
Pods number /plant	51.70	51.47	105.56	99.11
Pods weight / plant (g)	44.54	44.51	91.62	99.85
Seed yield/plant (g)	29.95	29.87	61.39	99.52
100-seed weight (g)	71.15	70.86	145.38	99.19
Chlorophyll content	14.59	14.37	29.15	96.99

Results considered Giza 834 and Giza716 cultivars were suitable genotypes to grow in arid or semi-arid regions. Results agreed with (Khatib *et al.*, 2019), who found that heritability in the broad sense was higher than 99.50% or nearing 100% of all estimated traits under both types of irrigation in barely genotypes. Jagadev *et al.*, (2021) and Magar *et al.*, (2021) reported that the reliable selection was done for characters with high PCV values, GCV levels, and h^2 % with GA, such as ear height, number of kernels, 100-kernel weight, and grain yield.

3.1.5. Tolerance indices

About 55.86% decrease in faba bean yield when plants are exposed to 50% FC (Table 3). Giza843 and Giza 716 cultivars had the highest values of MP, GMP and YSI while they had the lowest values TOL and values lower than unity for DSI indicate high-stress tolerance of them. These results agreed with Belal *et al.*, (2018), who reported that Giza-716 and Giza-843 cultivars appeared to be drought-tolerant genotypes. While Nubara1, Sakha-4, and Sakha-1 showed high TOL values and DSI values were more than unity, indicating that non-stress condition was a suitable environment to grow and produce the highest yield for these cultivars. Results were agreement with Bayoumi *et al.*, (2015); Afiah *et al.*, (2016); Khatab *et al.*, (2019) and Memari *et al.*, (2021) who reported DSI, TOL, GMP, MP and YSI indices had the most desirable selection criteria for high-yielding and screening drought-tolerant genotypes.

Table 3: Mean seed yield per plant (g) for two water treatments (100% FC and 50% FC), mean productivity (MP), geometric mean of productivity (GMP), tolerance index (TOL), yield stability index (YSI) and drought susceptibility index (DSI).

Genotypes	Grain Yield g/plant		MP	GMP	TOL	Ysi	DSI
	100% FC	50% FC					
Sakha 1	85.00	29.30	57.15	49.90	55.70	0.34	1.17
Sakha 4	105.30	31.70	68.50	57.78	73.60	0.30	1.25
Nubaria 1	100.00	34.00	67.00	58.31	66.00	0.34	1.18
Giza 716	90.00	50.00	70.00	67.08	40.00	0.56	0.80
Giza 843	100.00	67.00	83.50	81.85	33.00	0.67	0.59
RD %	55.86%						

3.1.6. Inter Simple Sequence Repeat (ISSR-PCR)

Results of ISSR-PCR showed varied in number and size of amplified products for primers; 14A, HB11, HB12, HB13, and HB15 (Figure 5). Thirty-seven amplified fragments were produced, with an average of 7.4 per primer (Table 4) which ranged between 4 to 11 for 14A primer and HB12 primer, respectively. the largest Alleles size rang 220-1745 were obtained from HB12 primer while the lowest one 215-445 were obtained by 14A primer. Heterozygosity /locus as ranged from 0.736 to 0.892 with an average of 0.827. Fourteen amplified fragments were polymorphic bands with an average 37.83% of Polymorphic percent, ranging from 0 to 54.54% for 14A primer and HB12 primer, respectively. Polymorphism information content (PIC) values were ranged from 0.75 to 0.89 with an average 0.84. polymorphism percent by the ISSR technique was enough to distinguish among different studied genotypes of faba bean. Results were in accepted with Abdel-Razzak *et al.*, 2012, Nurmansyah *et al.*, (2020) and Qahtan *et al.*, (2021) who reported that the diversity of genetic resources could be enriched in any crop by identifying and detecting new polymorphic bands or new alleles via genetic diversity studies.

Ten unique bands were obtained from three primers: HB12, HB11, and HB13. Six of them were obtained by HB12 primer which divided into three positive bands (1745bp, 1455bp, and 1195bp) found in Nobarey1 and three negative bands (380bp, 340bp, and 220bp) found in Giza716. The rest unique bands were negative obtained from HB11 primer and HB13 primer each of them produced two unique negative bands in Nobarey1. Results were agreement with Akladious and Abbas 2014 and Khatab *et al* 2019 who reported that to carry efficient and precise selection for resistance against biotic and abiotic stresses unique bands were produced by ISSR primers used as molecular markers in breeding programs

Values of similarity (Table 5) were ranged from 0.745(between Giza716 and Nobarey1) to 1(between Sakha 4 cultivars and Sakha1 cultivars). So, carrying hybridization between distant cultivars of this study, such as Giza716 and Nobarey1, might be a suitable method for improvement programs. These results were in harmony with Salazar- Laureles *et al.*, (2015), who found a genetic similarity coefficient ranging from 0.38 to 0.83 for 39 faba bean accessions suggesting wide genetic variability between accession at the molecular level.

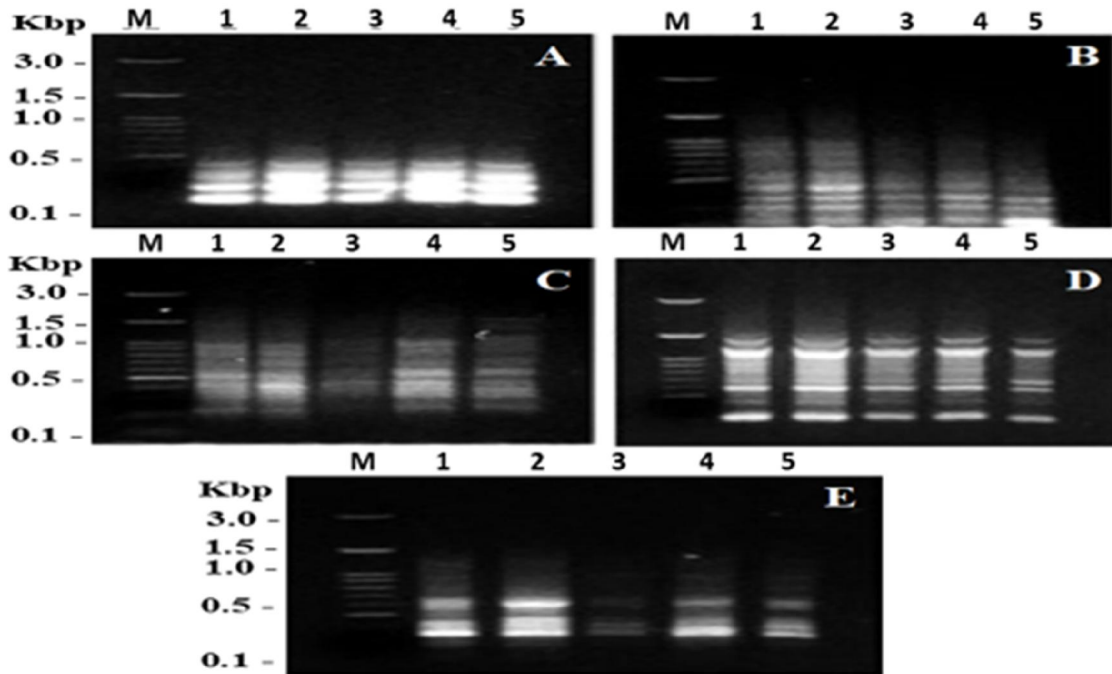


Fig. 5: ISSR profiles of the five cultivars by primers: 14A (photo A), HB-11 (photo B), HB-12 (photo C), HB-13 (photo D) and HB-15 (photo E). Lanes 1 to 6; M: DNA molecular weight marker, 1: Sakha1, 2: Sakha4, 3: Giza716, 4: Giza843, and 5: Nobarey1.

Table 4. Primer name, total number, monomorphic, polymorphic, unique bands, percent of polymorphism Numbers and size of the amplified fragments (bands) generated by ISSR primers, heterozygosity per locus values and polymeric information content (PIC) for each primer.

Primer Name	Total bands	Monomorphic Band	Polymorphic band	Unique Band	Polymorphic %	Allele's size rang	Heterozygosity per locus	PIC
14A	4	4	-	-	-	215 – 445	0.736	0.75
HB-11	8	4	4	2	50%	220 – 930	0.86	0.89
HB-12	11	5	6	6	54.54%	220 – 1745	0.892	0.89
HB-13	9	7	2	2	22.22%	320 – 1430	0.871	0.88
HB-15	5	3	2	-	40%	300– 865	0.78	0.79
Total	37	23	14	10	37.83%		4.139	4.2
Mean	7.4				37.83%		0.827	0.84

Table 5: Values of genetic similarity among five cultivars of faba bean based on ISSR analysis

	Sakha1	Sakha4	Giza 716	Giza 843	Nobarey1
Sakha1	1.0				
Sakha4	1	1.0			
Giza716	0.90566	0.90566	1.0		
Giza843	0.92593	0.92593	0.90196	1.0	
Nobarey1	0.81481	0.81481	0.7451	0.84615	1.0

Results of the dendrogram based on values of similarity divide studied genotypes into three main clusters (Figure 6). The first one contains Nobarey-1 cultivars only, while the second group includes three genotypes divided into two sub-clusters; one of them contains Sakha 1 and Sakha 4 cultivars and the second sub-cluster contains Giza843 cultivars, while Giza716 cultivar was found in the third cluster. The grouping of cultivars shows the degree of relatives among them according to their origin. Results were in harmony with Abdel-Razzak *et al.*, (2012) reported that the genetic diversity depending on genetic similarity for ten Egyptian faba bean genotypes using ISSR grouped into individuals. Results were in agreement with Asfaw *et al.*, (2018) and Wang *et al.*, (2012), who reported that ISSR primers grouped faba bean varieties into three distinct clusters and showed relationships among faba bean varieties. Either more Qahtan *et al.*, (2021) reported that resources of the faba bean from all continents were closely linked to their geographical origin.

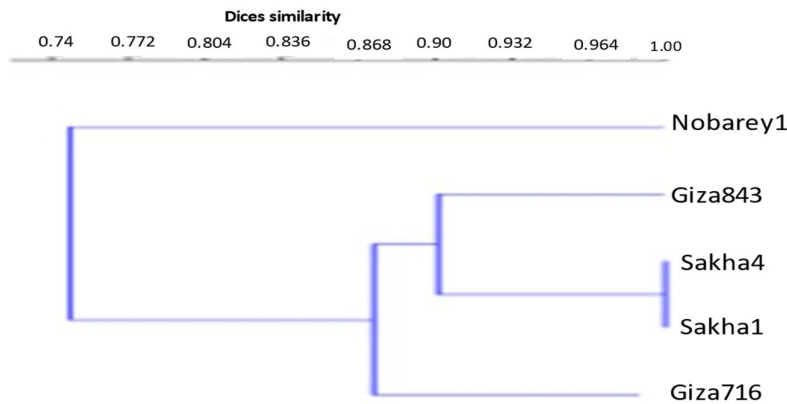


Fig. 6: Dendrogram cluster of five faba bean cultivars constructed by ISSR PCR primers data.

4. Conclusion

Five faba bean cultivars were studied for growth and yield parameters under three FC%. They revealed significant variation in grain yield and attributing traits. PCV was greater than GCV in all traits, implying that the environment had an impact. Some traits such as 100 seeds weight, leaves, and pods number /plant, pods weight/ plant might be used in successive selection programs for drought tolerance because they had the highest GCV, PCV, and heritability in a broad sense GAM values. Genetic variation between some studied cultivars gives a chance to use these materials through a hybridization process with other germplasm resources. ISSR-PCR techniques are suitable for detecting molecular markers for drought tolerance in faba bean cultivars. Ten unique bands are obtained and used as molecular markers in future breeding programs.

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