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Assessment of Spontaneous Variabilities and the Relation with Inferior of Yield and Quality Characteristics in Egyptian Cotton

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ABSTRACT

Yield and fiber quality characteristics are among the basic goals that the breeder seeks to maintain. The uniformity and homogeneity of these characteristics represents the practical criteria for identification and judging the purity of the cotton varieties. Maintaining genetic purity among cotton genotypes provides some protection against declining of yield potentials. The spontaneous genetic changes were assessed among Giza 86 and Giza 94 standard cotton cultivars and their off-types at Sakha Agric. Res. Stat. - Kafr EL Sheikh, Agric. Res. Center, Egypt, during 2021-2023. Significant differences were observed between the original parents either Giza 86 or Giza 94 and their derived off-types because of significant differences among original parents vs. off types. The off-types showed inferior values in most yield and fiber traits, decreased in lint percentage, fiber length with coarser and weaker lint and changed in lint color, light, radish to dark creamy lint. The differences among the standard cultivars Giza 86, Giza 94 and their off-types were mainly affected by two factors, the first factor was due to the cultivars and their off types, and the second factor was concerning the ability of characters that might exhibit discrimination, While ISSR primers flanked 68 loci in Giza 86 and their off-types with an average 9.71 bands per primer and 61.76 % polymorphism, While the most important difference revealed SSR primers was a repeat of the band within the genotypes.

INTRODUCTION

Yield and quality characteristics are among the basic goals that the breeder seeks to maintain. At the same time the uniformity and homogeneity of these characteristics represents the practical criteria for identification and judging the purity of the cotton varieties. Maintaining genetic purity among cotton genotypes provides some protection against declining of yield potentials.

The international fame of Egyptian cotton has been achieved due to its unique technological and spinning specifications, such as length, durability, and softness, in addition to the high degree of homogeneity and similarity of these qualities, which made international spinning mills prefer it over other cottons. This in addition to reducing losses during manufacturing, thus reducing production costs with high quality product, which ultimately increases competitiveness.

The propagation areas of cotton seeds grown annually are exposed to many causes of variation such as mechanical and genetic mixing factors that affect the genetic purity of the cultivated varieties which ultimately leads to the emergence of strange, hybrid patterns with poor, undesirable characteristics that ultimately affect the genetic purity of Egyptian cotton varieties (Hemaida *et al.*, 2006 and El-Mansy *et al.*, 2019) and ultimately some off-types existed that are spontaneously separated through the late segregations (Abd El-Salam *et al*., 2015).These variations include changes in seed characteristics, naked and fuzzy seeds, or / and lint color from brown to reddish with lower quality, shorter, weaker and coarser lint (El-Mansy *et al*., 2019) with late in maturity and decrease in lint percentage with very (El-Mansy *et al*., 2008 and Abd El-Salam *et al*., 2015). This may lead to deterioration of the Egyptian cotton and the markets rejection of these varieties, if they are randomly propagated (Ramadan, 2015).

In cotton, molecular markers were utilized to evaluate genetic diversity and relationships inside species and between relatives wild. Environment affects polygenic morphological markers, which are primarily quantitatively inherited (Lukonge *et al.*, 2007). Inter Simple Sequence Repeat (ISSR) has been applied in many genetic diversity studies. ISSR is a simple and informative genetic marker system in Cotton for revealing inter- and intraspecific variation (Abdellatif *et al.*, 2012 and Farahani *et al*., 2018). Simple sequence repeats (SSRs), or microsatellites, are a group of tandemly repeated DNA sequences comprised of one to six nucleotide units. These are ubiquitous in the genomes of prokaryotic and eukaryotic organisms (Iqbal *et al.*, 2001). Due to their greater polymorphism, SSRs are considered as an important marker system in fingerprinting, analysis of genetic diversity, molecular mapping and marker assisted selection (Reddy *et al*., 2001). The availability of SSR markers in the cotton genome make them useful in study of genetic diversity (Zhang *et al*., 2008). SSR

and ISSR markers are more strong, dependable, rapid, efficient, and reproducible than other approaches, with stronger discriminative ability (Dongre *et al*., 2007 and Preetha & Raveendren, 2008).

Therefore, this investigation aimed to assessment of spontaneous variabilities and the relation with inferior of yield and quality characteristics in Egyptian cotton.

MATERIALS AND METHODS

1. The aim, design and setting of the study

The importance of this study as one of the main research points in maintenance of the genetic purity for the Egyptian cotton. Canonical discriminate analysis and cluster analysis were used to study the differences among the original cultivar Giza 86, Giza 94 and their off-types based on morphological and molecular markers and to study the dangerous effect of such changes if they haphazard propagated in later generation.

2.The characteristics of participants or description of materials

The present experiment was done at Sakha Agricultural Research Station, Kafr EL Sheikh Governorate, Agricultural Research Center, Egypt, during 2021 , 2022 and 2023 growing seasons. Two Egyptian cotton cultivars, Giza 94, Giza 86 with their off types, four off-types for each, were used in this study. In the first season selfed seeds of both original cultivars and their off-types were sown and crossed to obtain eight F_1 seeds, four F_1 for each cultivar. In the second season F_1 seeds of the crosses Giza 94 and Giza 86 with dark creamy lint off-types were sown and selfed to obtain F_2 seeds for each cross.

For studying the genetically changes and the danger effects on yield and lint characteristics, a randomized complete block design (RCBD) with three replicates was done. Each replicate consisted of 9 rows for each group one row for each original cultivars, four rows for off-types and four rows for each F_1 crosses were sown in the second season. At the end of season, five plants for each entry were separately harvested and gained. Data were recorded on seed cotton yield/plant (g) (SCY/P), lint cotton yield/plant (g) (LCY/P), boll weight (g) (Bw), seed index (g) (SI), lint percentage (%) (LP %), lint index (g) (LI), micronaire reading (MR), fiber length (mm) (FL), uniformity index (UI), fiber strength (g/tex) (FS), pressley index (PI), lint reflectance (%) (Rd %) and degree of yellowness (+b). All fiber properties were measured in the laboratories of the Cotton Technology Research Division, C.R.I.

In 2023season, seeds of the F_1 and their F_2 population along with their parents of the crosses (Giza 86 x off type and Giza 94 x off type) were sown in no replication.

3. DNA Extraction for Polymorphism Analysis

Total genomic DNA isolated from leaves by protocol for rapid DNA isolation from cotton according to (Ali *et al*., 2019). The quantification and qualification of the extracted DNA was determined on 0.8 % agarose gel. The concentration and purity of extracted DNA were measured using a Nano drop spectrophotometer (BioDrop LITE.UK). Samples with purity of 1.8 or more were considered acceptable for PCR amplification. The amount of DNA template was 100 ng per 25-µl reaction volume.

3.1. Inter Simple Sequence Repeats (ISSR) Polymorphism Analysis

A set of seven primers (Table 1) were used for ISSR-PCR. The selected primers were recruited in PCR amplification reactions according to instruction supporting with MyTaq™ Red Mix, 2x.BIOLINE. Amplification was programed for 5-min initial denaturation step at 94C° followed by 35 cycles of denaturing at 94 C° for one min, annealing at 43 -52 C° (according to each primer) for one min, extension step at 72 C° for 1 min and a final extension step at 72 C° for 5 min by using (MyGene®–MG96G) programmable thermal cycler.

3.2. SSR analysis

Ten primer pairs specific for cotton microsatellite (SSR) used in these analyses were obtained from the Cotton Microsatellite Database (https://www.cottongen.org/search/markers) (Table 2). SSR-PCR amplifications were performed using MyTaq™ Red Mix, 2x.BIOLINE. Amplification was programmed for 5-min initial denaturation step at 94C° followed by 35 cycles of denaturing at 94 C° for one min, annealing at 45 - 48 C° (according to each primer) for one min, extension step at 72 C° for 1 min and a final extension step at 72 C° for 5 min by using (MyGene®–MG96G) programmable thermal cycler. The amplification products were electrophoresed against DNA ladder (250 bp and 10000 bp) to estimate the molecular sizes of the amplified fragments.

3.3. Data analysis and phylogenetic tree construction

Separated bands were scored and analyzed based on the presence and absence of bands (1 and 0) using the PAST program, version 1.90. The genetic similarity (based on Jaccard`s formula) was detected to establish genetic relationships among the investigated varieties based on unweighted pair group method of arithmetic averages (UPGMA) (Sneath & Sokal, 1973), using Past software (version 2.17) designed by (Hammer *et al*., 2001).

Table 1: The ISSR primers codes and Sequences for detecting polymorphism among all cotton genotypes.

Primer codes	Number of nucleotides	Sequence (5` to 3`)	Annealing temperature(ºC)
ISSR 156	17	AGCAGCAGCAGCAGCGA	51
ISSR 157	17	AGCAGCAGCAGCAGCGT	51
ISSR 158	17	AGCAGCAGCAGCAGCGC	52
ISSR 159	16	CACACACACACACAAC	43
ISSR 160	16	CACACACACACACAAT	41
ISSR 161	16	CACACACACACACAGA	43
ISSR 162	16	CACACACACACACAGC	45

Table 2: The SSR primers codes and Sequences for detecting polymorphism among all cotton genotypes.

Primer codes		Primer's sequence	Annealing temperature (ºC)
HAU0003	F	CGACTGCACTTAACTGTTGC	46
	R	GAACGATGAAATGGTTTTGG	
HAU0004	F	CCTGTGTTTTGATTTGATGG	45
	R	GTGACGATGAACCCACTACC	
HAU0005	F	CTCCACAATCAACAACTTCC	47
	R	CAGCCCATATGATAGTGAAGC	
HAU0006	F	GTTTTGGATCCACTTCAAGG	47
	R	GGTCGAAGTCATCCTCACC	
HAU0008	F	TCAACATCTCACCAACAAGC	48
	R	CATACTTGCAATTAGGACAAGC	
HAU00012	F	AATCTTCACTTTGTGGAGTCG	48
	R	TTTAGACCCCAAACTTCAGG	
HAU00010	F	GGTAATCGTGGTGTTTCTCG	45
	R	CATAGAAAATGATGCACACG	
HAU0016	F	TGCTGATGATTCTGATGTGG	53
	R	CAGCTTCTTTGGCTTTTAGC	
HAU0016	F	TTCATTGGCTGTGTACTTGG	53
	R	ACAGCGATTTCCTTTACTGC	
HAU0042	F	AATTTGGAGTGCAAGAGAGC	51
	R	AAGGGAAACAACAAACAAGG	

4. Statistical procedure

The studied traits were statistically analyzed on plot mean basis. A separate analysis of variance for each genotype was done to detect the significance of the observed differences. After this step, multivariate technique was conducted by using canonical discriminate analysis (Haire *et al*., 1987). This is a dimension-reduction technique related to principal component analysis and canonical correlation. It facilitates differentiation of groups by considering the interrelationships of the independent variables (traits) and the dependent (genotypes). An important property of canonical variables is that they are uncorrelated even though the underlying quantitative variables may be highly correlated. Hierarchical clustering was then carried out on each data set using Ward's minimum variance method, which minimize within cluster sum of squares. The results from clustering analysis are presented as dendrograms. The dendrogram is constructed on Euclidean distance basis according to (Nei, 1973) and developed by (Johnson & Wichern, 1988). All these computations were performed by using SPSS Computer Procedures (1995). All gels of molecular markers were scored as 0/1 for absence / presence of the bands, respectively and the resulting scored band were analyzed using PAST program according to (Hammer *et al*., 2001). The data matrix was used to calculate genetic similarity based on Accord's Similarity Coefficients to establish genetic relationship among the genotypes based on unweighted pair group method of arithmetic averages (UPGMA) and sequential agglomerative nested clustering.

RESULTS AND DISCUSSION

1. Analysis of variance

Analyses of variance for all studied traits are shown in Tables 3 and 4. The data showed significant mean squares of genotypes and parents in both Giza 86 and Giza 94 with their off-types for all studied traits except for boll weight and seed index of Giza 86 indicating the presence a lot of genetic variability. Significant differences were observed between the original parents either Giza 86 or Giza 94 and their derived off-types as a result of significant differences among original parents *vs* off types. The off-types showed differences from each other for some traits especially lint quality properties indicating that such changes appeared to be genetically alternations. Similar results were obtained by (Hemaida *et al.*, 2006 and El-Mansy *et al.*, 2008). F_1 crosses mean squares showed significant differences for fiber uniformity and degree of yellowness.

2. The mean performance of genotypes

The data in Tables 5 and 6 showed that the original cultivars either Giza 86 or Giza 94 surpassed off-types for all studied traits. The off-types gave inferior values in most yield and fiber traits, decreased in lint percentage and decreased in fiber length with coarser and weaker lint. Lint percentage is considered one of the important characteristics of cotton breeders, which are relied upon in selection, whether to produce new varieties and hybrids or the modernization of inbreed lines and the production of breeder and foundation seeds for cultivated commercial varieties. Therefore, any decline or deterioration of the varieties grown on a commercial scale because of the emergence of different off-types shows its effect in a decline in the ginning rate and consequently a decrease in the lint yield, which leads to huge losses for both producers and traders (Ramadan, 2015).

Most off-types showed inferior in fiber uniformity accompanied with short fiber. Fiber uniformity is very important for consumer market of cotton, since the higher index, the lower the losses in spinning processes (Araujo *et al.*, 2012). Some off-types showed changed in lint color, light, radish to dark creamy lint (Tables 5 and 6). This was undesirable phenomenon in cotton production since uniformity in lint color is one of the main objects of cotton breeders in Egypt. Lack of color uniformity was essentially responsible for market rejection of several Egyptian cotton varieties (El-Mansy *et al.*, 2019).

It could be concluded that the behavior of most off-types either in Giza 86 or/and Giza 94 were approximately similar. Such similar behavior was clearly pronounced for most traits, and the other off-types showed differences in lint color. This variability may be due to the accumulative number of plus modifiers which are high in creamy fibers. (Abo-Arab *et al.*, 2000) noticed that the creamy lint off-types had negative effect on lint yield and fiber quality characters.

3. Canonical discriminate functions analysis

Since the previous results assured the differences among the original cultivars Giza 86, Giza 94 and their off-types as well as F_1 crosses. These differences were mainly affected by two factors, the first factor was due to the cultivars and their off-types, and the second factor was concerning the ability of characters that might exhibit discrimination (Hemaida *et al*., 2006). Thus, canonical discriminate analysis simultaneously examines differences in the morphological variables and indicates the relative contribution of each variable to cultivar discrimination (Vaylay & Santon, 2002). Multivariate procedures based on, morphological and agronomic characters have been used in assessment of genetic variability and genetic diversity among original cultivars Giza 86, Giza 94 and their spontaneous off-types. In an analysis with 13 variables, there were 13 functions. However, only those that showed multivariate differences were considered. The first four and five functions were significant ($P < 0.01$) and accounted for approximately 99.6% and 98.8% of multivariate variation among all genotypes of Giza 86 and Giza 94, respectively (Tables 7 and 8).

Each canonical variate (genotypes) is the linear combination of independent variables (traits) and is orthogonal to the other. Thus, the maximal amount of variation is shown in the first function, the highest Eigen values were recorded in the first function and the second in the second function, this value could measure the explained variance associated with each variable (Haire *et al.*, 1987). Canonical correlation measures the strength of the overall relationships between the linear composites of predictor (canonical discriminate variate, characters, and criterion of predictor, genotypes, sets of variables). The significant ($P < 0.01$) canonical correlation between the genotypes with the first six canonical indicated that the canonical variate can explain the differentiation of genotypes. Similar results were reported by (El-Mansy *et al.*, 2008 and Abdel Salam *et al.*, 2015).

Canonical loadings measure the simple linear correlation between the traits and the functions, genotypes. Thus, the canonical loading reflecting the variance that the observed variables share with the canonical variate, and it can be interpreted in assessing the relative contribution of each variable to each canonical function (Haire *et al.*, 1987). Thus, each character was an important source of variance in, at least, one discriminate function, and some characters may have greater importance in determining plant phenotypes than others (El-Mansy *et al.*, 2012).

Table 3: Mean squares of yield components and fiber quality characteristics for Giza 94.

S.O.V.	d.f.	SCY/P (g)	LCY/P (g)	BW (g)	SI (g)	LP %	LI (g)	MR	FL (mm)	UI	FS (g/tex)	PI	Rd	+b
Replications	2	60.40	9.96	0.003	0.357	0.818	0.194	0.019	2.548	5.311	2.983	0.154	3.796	0.037
Genotypes	8	515.43*	113.29*	0.528*	2.241*	20.324*	3.574*	0.277*	9.323*	26.448*	14.461*	1.041*	29.137*	6.942*
Parents	4	558.76*	187.07*	0.839*	3.972*	38.554*	6.891*	0.399*	14.462*	36.707*	26.626*	1.831*	56.354*	11.788*
Off-types	3	183.56	22.736	0.194	2.07	0.419	0.651	0.092*	4.203*	9.274	6.38*	0.661*	10.459	6.829*
Original parents vs off-types	1	1684.34*	680.06*	2.774*	9.680*	152.960*	25.611*	1.320*	45.240*	119.004*	87.363*	5.340*	194.04*	26.667
Crosses	3	341.2	33.88	0.179*	0.68	0.629	0.301	0.0156	1.128	20.523*	2.174	0.184	2.490	2.774
P. vs C.	1	864.9*	56.9*	0.333*	0.0003	6.49*	0.128	0.574*	13.349*	3.189	2.559	0.451	0.208	0.056
Error	16	95.88	12.07	0.048	0.337	0.914	0.113	0.0113	0.479	3.091	0.775	0.087	2.259	0.346

* & ** are significant levels at 0.05 and 0.01 levels of probability, respectively.

Table 4: Mean squares of yield components and fiber quality characteristics for Giza 86.

S.O.V.	d.f.	SCY/P (g)	LCY/P (g)	BW (g)	SI (g)	LP %	LI (g)	MR	FL (mm)	UI	FS (g/tex)	PI	Rd	+b
Replications	2	47.8	12.37	0.055	1.221	1.378	0.908	0.023	1.874	1.830	2.484	0.099	1.668	0.227
Genotypes	8	283.1*	158.27*	0.091	0.278	22.986*	1.693*	0.192*	7.500*	46.324*	37.448*	1.586*	41.275*	5.827*
Parents	4	525.1*	313.22*	0.161	0.167	39.498*	3.103*	0.338*	10.629*	66.389*	68.178*	2.889*	73.348*	10.004*
Off-types	3	188	22.47	0.081	0.016	0.392	0.176	0.112	1.532	10.111*	21.463*	1.114	29.100	2.923*
Original parents vs off-types	1	1536.2*	1185.48*	0.400	0.620*	156.82*	11.882*	1.014*	37.921*	235.224*	208.32*	8.214*	206.091*	31.248*
Crosses	3	53.70	0.832	0.023	0.229	5.734	0.366	0.014	1.543	10.517*	3.583	0.252	12.269	2.185*
P. vs C.	1	3.09	10.753*	0.0125	0.864	8.69	0.033	0.140	12.849*	73.483*	16.12*	0.373	0.002	0.041
Error	16	106	12.92	0.079	0.484	2.314	0.336	0.033	1.331	2.016	1.792	0.418	4.656	0.240

* & ** are significant levels at 0.05 and 0.01 levels of probability, respectively.

Table 5: Means of yield components and fiber quality characteristics for Giza 94, its four off-types (T) and their F_1 crosses.

Genotypes[a]	SCY/P (g)	LCY/P (g)	BW (g)	SI (g)	LP %	LI (g)	MR	FL (mm)	UI	FS (g/tex)	PI	Rd	+b
Giza 94	105.93	42.20	4.20	12.03	39.87	7.97	4.07	34.73	85.83	42.07	10.47	76.40	7.80
T1	88.40	28.57	3.20	10.60	32.27	5.07	4.90	29.17	76.77	37.53	8.67	67.87	11.60
T2	78.67	24.90	3.17	9.43	31.63	4.37	4.97	29.63	77.90	34.03	8.57	64.67	12.97
T3	69.47	21.93	2.77	9.20	31.50	4.23	4.57	31.10	80.50	36.33	9.60	68.80	9.43
T4	81.23	26.07	3.37	10.87	32.13	5.13	4.80	31.67	80.00	36.23	9.07	68.30	10.53
F_1-1	61.73	22.47	2.83	10.73	34.73	5.73	4.33	32.63	81.43	36.47	9.37	68.50	10.10
F_1-2	67.77	23.80	3	9.73	34.17	5.03	4.47	33.43	76.93	37.73	9.47	68.80	11.83
F_1-3	78.27	27.10	3.37	10.73	34	5.53	4.30	31.93	82.07	36.57	9.40	70.50	9.60
F_1-4	85.63	29.93	3.27	10.53	34.97	5.67	4.37	32.70	77.60	35.67	9.90	69.73	10.70
LSD 0.05	16.45	5.90	0.36	1.00	1.63	0.60	0.19	1.44	3.13	1.73	0.53	2.67	0.96
LSD 0.01	22.54	8.08	0.49	1.37	2.23	0.82	0.26	1.98	4.29	2.37	0.72	3.66	1.31

[a] Genotypes indicates the original cultivar Giza 94, its four off-types (T1, T2, T3 & T4) and their four F_1 crosses.

Table 6: Means of yield components and fiber quality characteristics for Giza 86, its four off-types (T) and their F_1 crosses.

Genotypes[a]	SCY/P (g)	LCY/P (g)	BW (g)	SI (g)	LP %	LI (g)	MR	FL (mm)	UI	FS (g/tex)	PI	Rd	+b
Giza 86	92.30	43.60	3.33	10.93	39.77	7.20	4.37	33.53	86.40	44.73	10.73	76.40	8.20
T1	78.33	25.27	2.90	10.40	31.50	4.80	5.07	29.40	76.73	35.67	9.07	70.33	10.97
T2	64.07	20.10	2.73	10.53	31.27	5.33	4.77	30.37	74.47	37.83	9.07	68.10	11.43
T3	65.60	21.13	3.13	10.40	32.03	4.90	5	29.80	78.87	36.53	9.40	67.20	11.60
T4	60	19	2.93	10.37	31.93	4.87	5.23	28.67	75.93	31.63	8	62.90	13.23
F_1-1	66.97	23.80	3.10	10.10	35.57	5.53	4.80	31.30	83.03	36.60	8.97	70.33	10.97
F_1-2	71.07	24.67	3.13	9.87	34.73	5.27	4.67	32.67	83.73	36.73	9.30	69.60	11.20
F_1-3	77.13	25.03	2.93	10.17	32.43	4.90	4.80	31.07	79.87	34.67	8.63	69.97	9.90
F_1-4	70.37	24.70	3.03	10.53	35.03	5.70	4.70	31.93	80.57	34.90	9.17	65.97	11.97
LSD 0.05	17.12	6.15	0.48	1.29	2.55	1.08	0.31	2.02	2.42	2.35	1.06	3.57	0.85
LSD 0.01	23.45	8.43	0.65	1.77	3.49	1.48	0.42	2.77	3.32	3.21	1.45	4.89	1.17

[a] Genotypes indicates the original cultivar Giza 86, its four off-types (T1, T2, T3 & T4) and their four F_1 crosses.

The first canonical discriminate function which represented 78.6% and 62.0% of the total variability among genotypes of Giza 86 and Giza 94 with their off-types with the largest Eigen value (64.01 and 74.0) respectively is dominated by a large loading from lint percentage, lint index followed by degree of yellowness and fiber reflectance among Giza 86 group. While, among Giza 94 group lint index, lint percentage followed by fiber fineness and degree of yellowness were the primary source of variation and showed high loading values (Table 8). The genotypes which possess high lint percentage and lint index values showed positive values of other traits with increasing micronaire and yellowness values (inferior values). The second function is largely affected by fiber strength, fiber length which showed positive loadings and micronaire reading (negative loading) accounted for about 14.0% of the total variance among Giza 86 group genotypes. The same trend was appeared among Giza 94 genotypes since the second function accounted about 22.5% of the total variance and a large dominated by most fiber properties. The third function was highly affected by LP% followed by boll weight and fiber strength. It also showed positive discrimination. The variances explained by the third and fourth functions were 5.5%, and 1.6%, respectively with Eigen values more than unity among Giza 86 group and largely affected by lint color and yield traits. The third, fourth and fifth functions accounted for about 10.6%, 2.1% and 1.5 of the total variance among Giza 94 group and were highly affected by fiber degree of yellowness and most yield traits.

It is clear that the genetic composition of the standard cultivars Giza 86, Giza 94 and their off-types differ mainly in some characteristics such as lint percentage, degree of yellowness, fiber length, uniformity of fibers, fiber strength, and micronaire reading. On the other hand, some characters showed greater discrimination than the others. These enable cotton breeder to predict and detect changes or deteriorations in cultivars when some of them were deviate from the standard types of characteristics such as lint percentage, lint color and other fiber properties. (Hemaida *et al*., 2006) explained that percentage of lint followed by lint index revealed the presence of high discrimination within Giza 83 and it's off-types, While (El-Mansy *et al*., 2008) found that lint color followed by fiber length and lint percentage explained high multivariate variance among Giza 70, Giza 89 and their off-types.

The nine genotypes, Giza 86, Giza 94 standard cultivars, four off-types from each and their F_1s were plotted according the first two functions (Figure 1). It is clear that the first function separated the standard cultivars Giza 86 and Giza 94 as a common parent at the separate group and widely distance from the other off-types and F_1 crosses, according to differentiation of characters which largely affected. While the off-types from each cultivar and their F_1s were separated by the second function. (Ramadan, 2015) noticed that the first and second functions were more effective to separate the standard cultivar Giza 88.

Table 7: Canonical discriminate functions analysis and Structure Matrix for Giza 86.

variables	function			
	1	2	3	4
LP %	0.524*	- 0.068	- 0.119	0.318
LI (g)	0.424*	- 0.025	0.116	0.262
BW (g)	0.254*	- 0.237	- 0.235	- 0.005
UI	0.304	0.419	0.456	0.531*
Rd	0.325	- 0.008	- 0.625*	0.001
FS (g/tex)	0.342	0.875*	- 0.039	- 0.192
PI	0.278	0.417*	- 0.128	0.170
SI (g)	0.228	0.120	0.444	0.152
+b	- 0.369	- 0.120	0.585	- 0.124
SCY/P (g)	0.126	- 0.180	- 0.144	0.034
LCY/P (g)	0.287	- 0.246	0.052	0.007
FL (mm)	0.117	0.281	0.143	- 0.029
MR	- 0.112	- 0.303	0.019	- 0.232
Eigen value	64.011[a]	11.408[a]	4.441[a]	1.279[a]
% of variance	78.6	14	5.5	1.6
Cumulative %	78.6	92.6	98.1	99.6
Canonical Correlation	0.992	0.959	0.903	0.749

Table 8: Canonical discriminate functions analysis and Structure Matrix for Giza 94.

variables	functions				
	1	**2**	**3**	**4**	**5**
SI (g)	- 0.132	0.348	0.062	- 0.141	0.623*
LCY/P (g)	0.112	0.274	- 0.050	0.089	0.592*
SCY/P (g)	0.231	0.323	- 0.088	0.086	0.538*
+b	0.280	0.128	0.460	0.161	- 0.518*
LI (g)	- 0.377	0.463	0.145	- 0.201	0.479*
BW (g)	- 0.175	0.433	0.208	- 0.254	0.115
LP %	-.0.353	0.336	0.133	- 0.059	0.056
FL (mm)	- 0.251	0.288	- 0.030	0.145	0.026
MR	0.331	- 0.333	0.275	- 0.131	- 0.135
UI	- 0.155	0.070	- 0.232	- 0.165	0
FS (g/tex)	- 0.248	- 0.471	0.146	- 0.109	0.475
Rd	- 0.268	0.229	- 0.401	0.077	0.273
PI	- 0.257	0.299	- 0.263	0.209	0.143
Eigen value	74.009[a]	26.851[a]	12.702[a]	2.496[a]	1.824[a]
% of variance	62	22.5	10.6	2.1	1.5
Cumulative %	62	84.6	95.2	97.3	98.8
Canonical Correlation	0.993	0.982	0.963	0.845	0.804

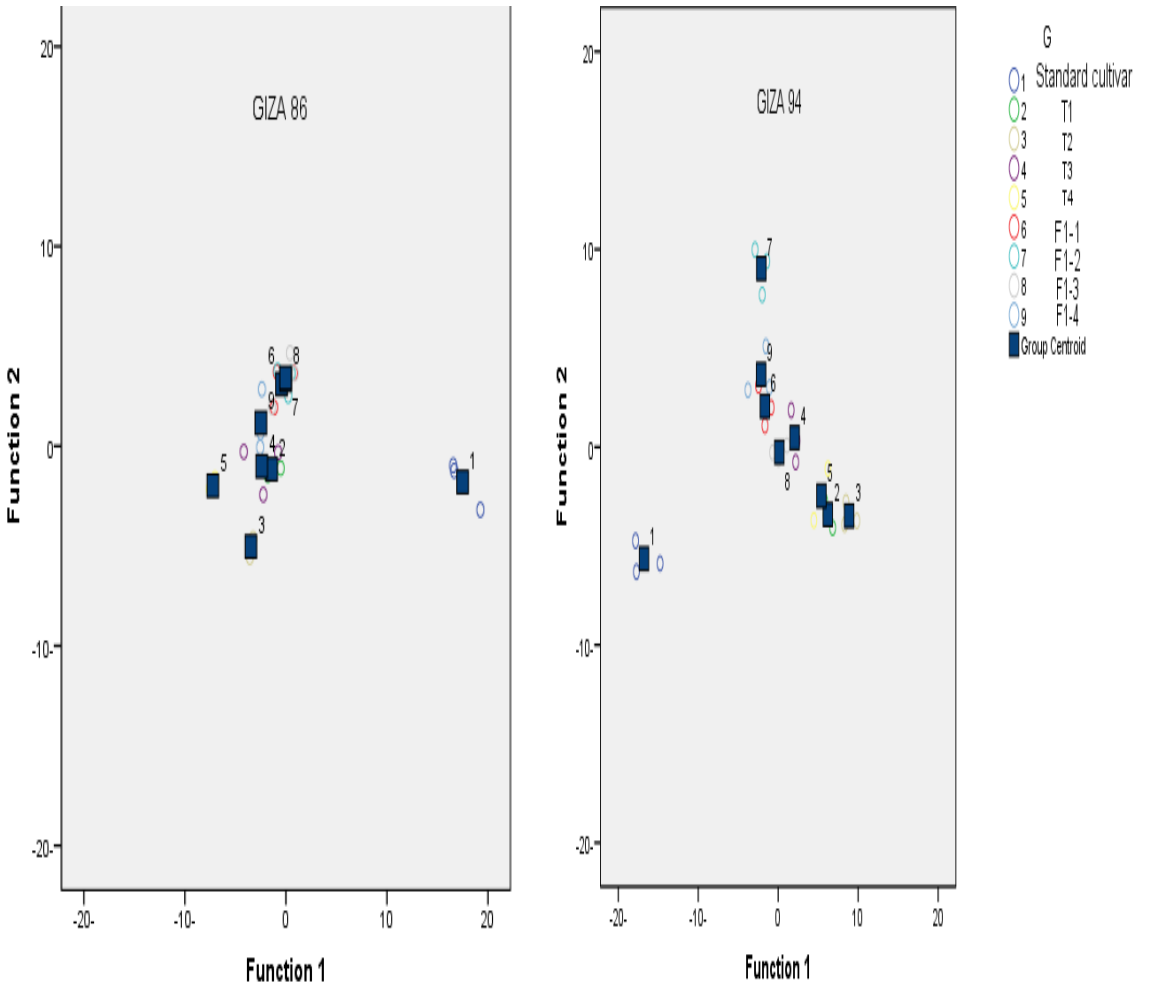

Fig. 1: The centroid values for the two canonical functions for Giza 86 and Giza 94, its four off-types (T) from each as well as their F_1 progenies.

The nine genotypes (standard cultivars Giza 86, Giza 94 and four off-types for each as well as F_1 progenies derived from crossing among them were grouped into five and four clusters according to hierarchical clustering analysis based on the relative dissimilarities among them and contribution the evaluated traits (Figure 2). It is clear that the standard cultivars Giza 86 or Giza 94 formed a unique groups and widely diverged distance from the other off-types as well as F_1 progenies (Table 9). It is clear evident that the four off-types in both varieties were grouped in varied clusters. Type's number 4, 2 in Giza 86 and type's number 2 and 4 in Giza 94 formed a wide group from the standard cultivars and the other off-types. This was true since these types differed widely and might assure the occurrence of double spontaneous alternations. Such alternation might be induced simultaneously in seed and lint color after some times. These results are in harmony with those obtained by (El-Mansy *et al.,* 2019).

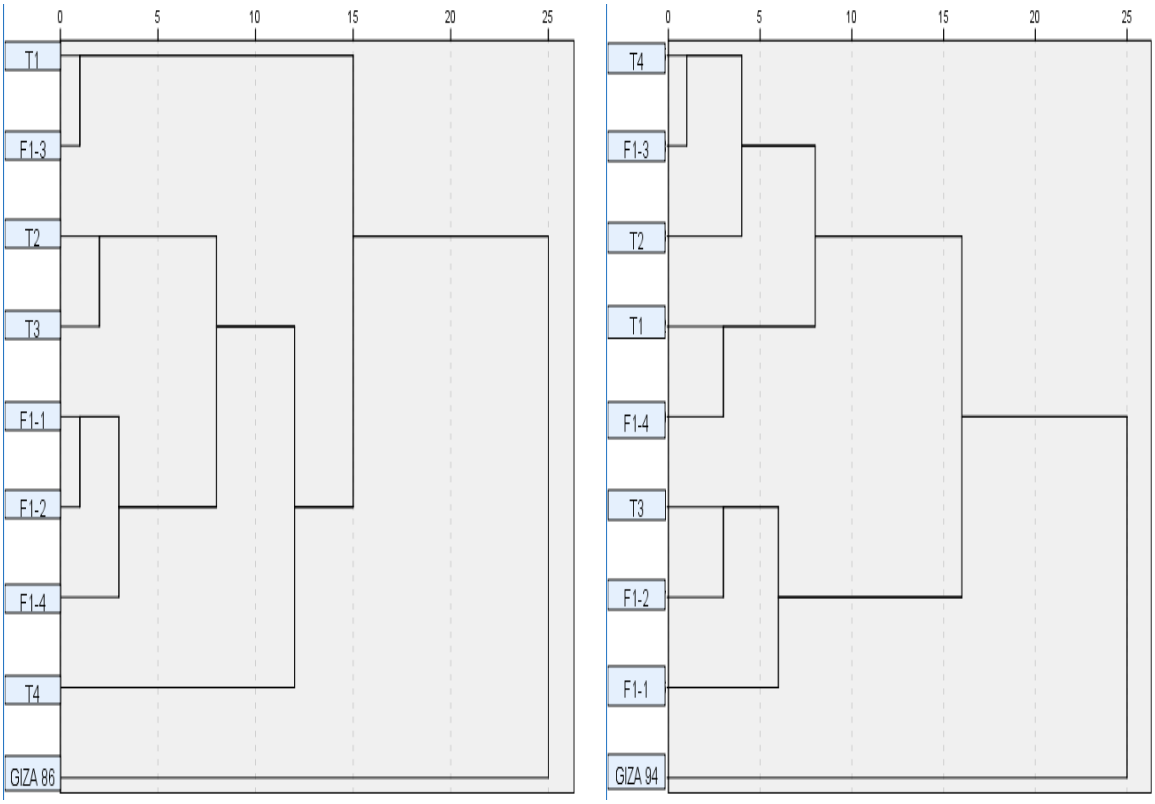

Fig. 2: Hierarchical clustering analysis of Giza 86, Giza 94, its four off-types (T) from each and their F_1 progenies.

Table 9: Distances between and within clusters for Giza 94, Giza 86 and their off-types from each as well as their F_1 progenies.

Cluster	Giza 94			
	1	**2**	**3**	**4**
1	0.00	27.618	34.718	46.264
2		3.195	8.909	21.882
3			4.40	13.698
4				4.1

Cluster	Giza 86				
	1	**2**	**3**	**4**	**5**
1	0.00	28.905	40.077	32.859	47.293
2		2.131	14.139	10.116	20.812
3			2.572	9.315	9.311
4				3.110	15.527
5					0.00

Finally, all plant breeders must have through knowledge about variability in their crop, and all have an intuitive feel for how different genetic groups relative to one another when considering many traits simultaneously. Generally, canonical discriminate function analysis is useful in identifying the genetic variation and the most influential traits affecting genetic variation of plant populations. (Vaylay & Santon, 2002) canonical loadings of morphological and agronomic traits of an individual cultivar indicate the magnitude of genetic variation. Knowledge of genetic variation of traits among various type of variability which existed spontaneously in the standard varieties in response to natural selective forces will be useful for plant breeders by focusing attention on such traits and could safety condense selection to eliminate such off-types easily from the original cotton cultivars.

The results of canonical discriminate function analysis and cluster analysis appeared to be of complete accordance. The canonical analysis could provide no clear grouping but gave a special idea for genetic variability and most influential characters however, cluster analysis could efficiently describe the characteristics of groups of different genotypes and both gave a sensible and useful integration of the data. However, more extensive molecular data are needed in order to interpret the best general conclusion about the relationship among the Giza standard varieties and their off-types.

Genetic consequence of the haphazard transfer of the off-types genes on lint percentage of both Giza 86 and Giza 94 cotton cultivars were studied in segregates' generation. Frequency distribution curves, ranges and means for lint percentage of F_2, F_1 and the parents are shown in Figures (3). The differences among the standard cotton cultivars and their off-types were clearly distinctive. The off-types showed lower lint percentage values as compared with standard cultivars. Distribution of F_1 tended to behave as their common parents exhibiting a case of slightly partial dominance towards their standard cultivars. (El-Mansy *et al.*, 2008) detected partial dominance controlling lint percentage in F_1 derived from crossing original variety Giza 70 and naked seed off type.

The F_2 frequency distribution curves were characterized by a sort of unimodality indicating the continuous type of variation for the studied trait, due to the joint action of polygene. The presence of transgressive segregation in negative direction (lower values) might due depression in later generation which cause a dangerous effects of such off-types.

The range, an index of variability, was comparatively wider in F_2 generation as compared with the parents for the studied traits. On the same time the lower limits of range were lower in F_2 generation leading to wider spectrum of variability. However, in standard cultivars the lower limits of range were relatively high and the upper limits were also relatively high. The means of F_2 populations behaved in the manner as their off-types parents and showed depression from the standard parents showing that the characters of such off-types could be easily transmitted to their progenies. Meanwhile, the differences between the F_1 and F_2 might indicate a case of inbreeding effect towards degeneration (Table 10).

The ginning rate is considered one of the important traits for cotton breeders, which are relied upon in selection and production of pure strains. Most previous studies have shown that this trait is controlled by the additive gene action with a high degree of heritability. Thus, the breeder could safety to condense selection for better lint percentage and lint quality values to eliminate any changes from the standard cotton cultivars for preventing their degenerations.

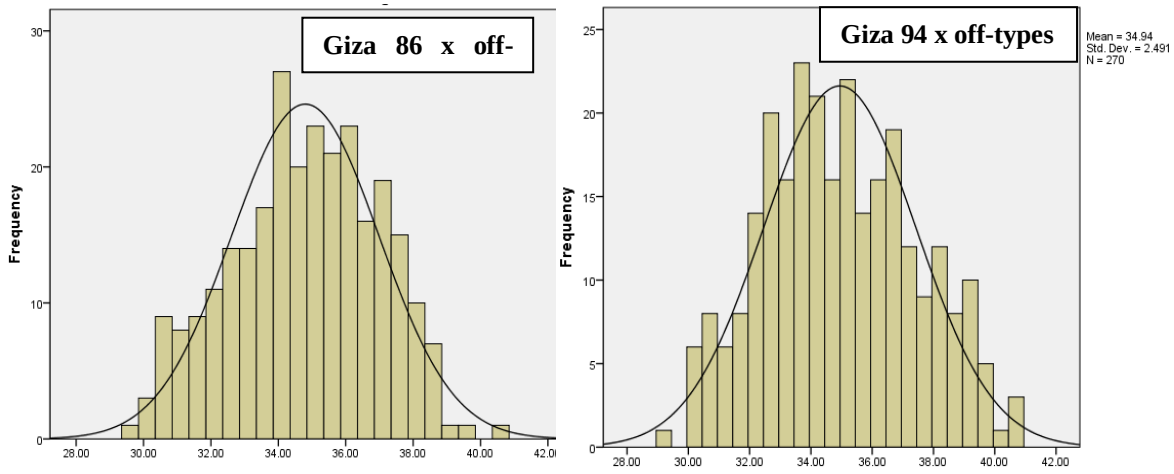

Fig. 3: Frequency distribution curves of the studied F_2 populations.

Table 10: Means, Range and standard deviation for Giza 94, Giza 86, four off-types from each and their F_1s and F_2s.

	Genotypes	Mean ± SE	Std. Dev.	Variance	Range	
					Maximum	Minimum
F_2	Giza 94 x off-type	34.94 ± 0.1516	2.4910	6.205	40.80	29.20
	Giza 86 x off-type	34.80 ± 0.1331	2.1877	4.786	40.60	29.60
P_1	Giza 94	39.72 ± 0.1360	0.7451	0.555	41.70	38.50
	Giza 86	38.87 ± 0.1238	0.6783	0.460	40.10	37.80
P_2	Off-type Giza 94	33.11 ± 0.1915	1.0487	1.100	34.90	31
	Off-type Giza 86	32.44 ± 0.1754	0.9604	0.922	34.70	29.80
F_1	Giza 94 x off-type	36.95 ± 0.1599	0.8756	0.767	38.60	34.90
	Giza 86 x off-type	36.64 ± 0.1476	0.8084	0.654	38	35.20

4. Inter Simple Sequence Repeats Polymorphism (ISSR) Marker

ISSR primers flanked 84 loci in Giza 94, its four off-types and their F_1 crosses (Table 11), out of them 14 were unique (marker bands). The other detected 43 loci were segregated loci (polymorphic), while 27 monomorphic loci were detected. An average of 12 bands per primer was amplified and 51.19% were Polymorphism. The most efficient ISSR markers were ISSR 156 and ISSR 157 which detected the highest number of loci; totally 32 loci distributed as unique, polymorphic, and monomorphic. The ISSR 158 and ISSR 160 primer detected 0 unique loci, 7 segregated loci and 5 monomorphic loci for ISSR 158. While ISSR 160 primer were detected 2 monomorphic and 10 polymorphic. The ISSR 159 primer detected 1 unique locus, 2 polymorphic loci and 8 monomorphic loci. Both of ISSR 161 and ISSR 162 primers had the least efficiency in flanking DNA fragments as it detected only 1 unique, 4 polymorphic and 3 and 4 monomorphic loci, respectively.

Table 11: Number of monomorphic fragments, polymorphic fragments and percentage of polymorphism obtained ISSR primer for Giza 94, its four off-types and their F_1 crosses.

Primers	Range of fragments size (bp)	Total No. of fragments	Monomorphic fragments	Polymorphic fragments	Unique fragments	Polymorphism %
ISSR 156	150-1300	16	2	7	7	43.75
ISSR 157	200-1500	16	3	9	4	56.25
ISSR 158	200-750	12	5	7	0	58.33
ISSR 159	300-1000	11	8	2	1	18.18
ISSR 160	200-500	12	2	10	0	83.33
ISSR 161	100-500	8	3	4	1	50.00
ISSR 162	500-650	9	4	4	1	44.44
Total		84	27	43	14	
average		12	3.85	6.14	2	51.19

Table 12: Number of monomorphic fragments, polymorphic fragments and percentage of polymorphism obtained ISSR primer for Giza 86, its four offtypes and their F_1 crosses.

Primers	Range of fragments size (bp)	Total No. of fragments	Monomorphic fragments	Polymorphic fragments	Unique fragments	Polymorphism %
ISSR 156	150-1000	11	2	8	1	72.72
ISSR 157	100-800	13	1	11	1	84.61
ISSR 158	250-700	10	3	7	0	70.00
ISSR 159	200-900	9	3	6	0	66.66
ISSR 160	100-500	9	5	4	0	44.44
ISSR 161	100-550	7	2	5	0	71.42
ISSR 162	200-650	9	8	1	0	11.11
Total		68	24	42	2	
average		9.71	3.42	6	0.28	61.76

ISSR primers flanked 68 loci in Giza 86, its four off-types and their F_1 crosses (Table 12), out of them 2 were unique (marker bands). The other detected 42 loci were polymorphic, while 24 monomorphic loci were detected. An average of 9.71 bands per primer was amplified and 61.76% were Polymorphism. The most efficient ISSR markers were ISSR 157 which detected the highest number of loci; totally 13 loci distributed as unique, polymorphic, and monomorphic with 84.61% Polymorphism. Except ISSR 156 and 157 all primers were detected 0 unique loci. The ISSR 162 primer had the least efficiency in flanking DNA fragments as it detected only 0 unique, 1 polymorphic and 8 monomorphic loci by 11.11 % polymorphism.

Genetic similarity among Giza 94, its four off-types and their F_1 crosses ranged from 0.50 to 0.88 (Table 13). The highest similarity detected by ISSR was between Giza 94 and its four off-types with mean similarity (0.72) while the least similarity was between Giza 94 and their F_1 crosses with mean similarity (0.56).

The dendrogram of genetic distances among Giza 94, its four off-types and their F_1 crosses based on band polymorphisms generated by ISSR-PCR after using the primers were shown in (Figure 4). The ISSR-phylogenetic dendrogram was divergent into two clusters; one of them divided into two sub-clusters included Giza 94 and its four off-types whose mean similarity was 0.72 and F_1-4 with genetic similarity equaled 0.66 represent group I. The other cluster was divided into two sub- clusters; one of them included F_1-1 and the other included of F_1-2 and F_1-3.

On the other hand, genetic similarity among Giza 86, its four off-types and their F_1 crosses ranged from 0.46 to 0.76 (Table 13). The highest similarity detected by ISSR was between Giza 86 and its four off-types with mean similarity (0.70) while the least similarity was between Giza 86 and F_1-hyprides with mean similarity (0.53).

The dendrogram of genetic distances among Giza 86, its four off-types and their F_1 crosses based on band polymorphisms generated by ISSR-PCR after using the primers were shown in (Figure 4).

Table 13: Similarity and distance indices among Giza 94 (above diagonal), Giza 86 (below diagonal), its four off-types (T) from each cultivar and their F_1 crosses according to ISSR pattern.

	Giza 86	**T1**	**T2**	**T3**	**T4**	**F_1-1**	**F_1-2**	**F_1-3**	**F_1-4**
Giza 94	--	0.72881	0.75	0.74194	0.68254	0.58667	0.50725	0.53623	0.63934
T1	0.69811	--	0.9	0.88462	0.81132	0.53521	0.59322	0.6	0.73077
T2	0.72	0.62264	--	0.83636	0.76786	0.49333	0.53968	0.54688	0.66071
T3	0.64	0.67347	0.59184	--	0.82143	0.57534	0.63934	0.59375	0.68421
T4	0.76364	0.67241	0.72222	0.53448	--	0.52703	0.53125	0.5625	0.59322
F_1-1	0.52542	0.60714	0.53571	0.64	0.56452	--	0.73438	0.68657	0.57353
F_1-2	0.46032	0.61404	0.54386	0.55556	0.59677	0.7037	--	0.78182	0.67273
F_1-3	0.53571	0.56364	0.67347	0.65957	0.57627	0.68627	0.62963	--	0.67857
F_1-4	0.60345	0.63158	0.58929	0.54545	0.69492	0.69091	0.75926	0.58929	--

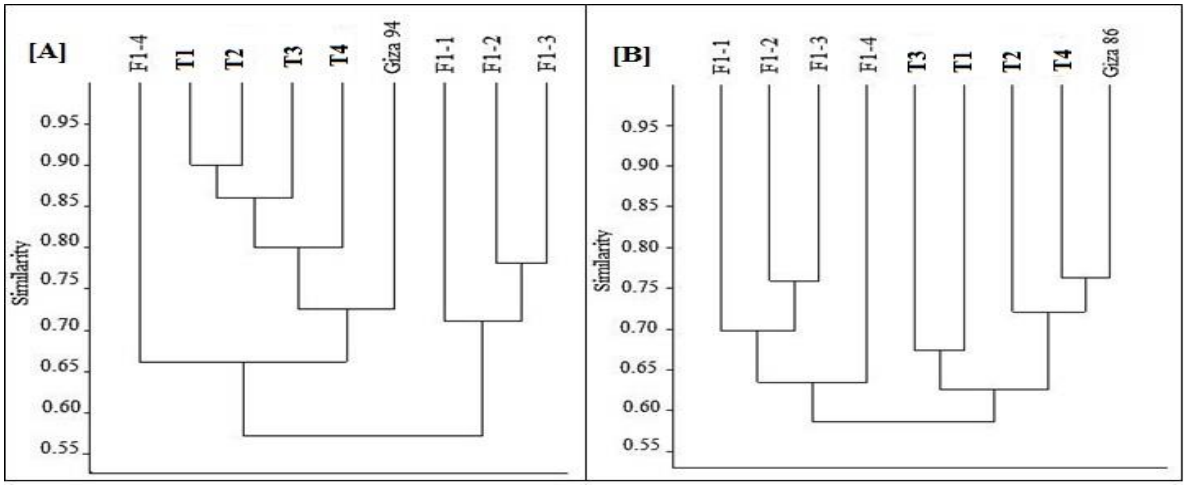

Fig. 4: UPGMA phylogenetic dendrogram representing the genetic distance for Giza 94, Giza 86, its four off-types (T) from each cultivar and their F_1 crosses according to ISSR pattern, using seven primers.

The ISSR-phylogenetic dendrogram was diverged into two clusters; one of them divided into two sub-clusters included Giza 86 and T4 with high similarity about 0.76 and other sub-cluster included T1 and T3 with genetic similarity equaled 0.67 represent group I. The other cluster was divided into two sub- clusters; one of them included F_1-4 and the other included of F_1-1 in sub-cluster while F_1-2 and F_1-3 in other. Therefore, the discrimination power of ISSR markers obtained in this study suggests that they could be used to examine the diversity of Cotton genotypes efficiently and precisely and encourage targeted crossing strategies.

Inter Simple Sequence Repeat (ISSR) has been applied in many genetic diversity studies. ISSR is a simple and informative genetic marker system in Cotton for revealing inter- and intraspecific variation (Abdellatif *et al*., 2012 and Farahani *et al*., 2018). ISSR markers have been used for differentiating cotton genotypes. For example, the cotton genotypes (*G. barbadense* L.) were clustered into two major clusters using a UPGMA cluster analysis based on ISSR polymorphism, according to (Hoffmann *et al*., 2018). In many studies on *Gossypium* genotypes and other plants, when compared to the different molecular markers utilized, ISSR markers produced the largest percentage of polymorphic bands (Liu *et al.,* 2006; Abdellatif *et al.,* 2012 and Jedrzejczyk & Rewers, 2020); it's believed that this is because ISSR is a dominant marker that measures the distance between two microsatellites.

5. Polymorphism analysis as detected by SSR

Ten primer pairs specific for cotton microsatellite (SSR) used to determine genetic diversity and phylogenetic relationships between the two varieties, its four off-types and their F_1 crosses. Each primer from ten primers used in this study showed one monomorphic band in all genotypes with slight variation in size of band between variety, off-types and their F_1 crosses. While the most important difference revealed SSR primers was a repeat of the band within the genotypes. Some SSR primer such as (HAU0003) showed an increase in repeats of the band in F_1 crosses as compared to off types, while HAU0004 primer showed decrease in repeats of the band in off-types compared to F_1. Figure (5) as an example of DNA profiles on agarose gel of Giza 94 and Giza 86 by two SSR primers. Genetic diversity comes from the allelic variation in the genome (duplication, insertion, deletions in DNA) and constitutes the basis of Marker-assisted breeding. The Simple Sequence Repeat (SSR) molecular markers were used in the genetic diversity analyses because they have a high ability to show genetic differences between cotton genotypes, they are present in all eukaryotic cells, show uniform distribution throughout the genome, provide the opportunity to determine genetic diversity, are repeatable, allow working on low DNA samples amount, are cheap and co-dominant, and give reliable results. SSR markers are in 1-4 to 1-6 nucleotide length (Abdalla *et al*., 2001 and Iqbal *et al*., 2001). Due to their greater polymorphism, SSRs are considered as an important marker system in fingerprinting, analysis of genetic diversity, molecular mapping and marker assisted selection (Reddy *et al*., 2001). The availability of SSR markers in the cotton genome make them useful in study of genetic diversity (Zhang *et al*., 2008).

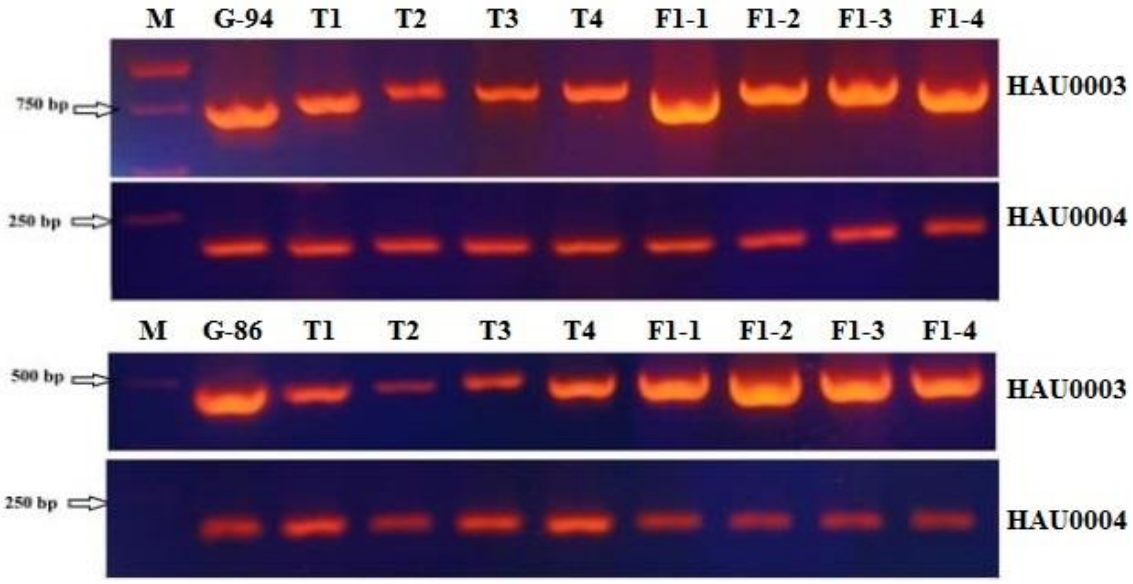

Fig. 5: DNA profiles of Giza 94, Giza 86, its four off-types (T) from each cultivar and their F_1 crosses by SSR primer

DISCUSSION

The off-types showed differences from each other for some traits especially lint quality properties indicating that such changes appeared to be genetically alternations. Canonical loadings measure the simple linear correlation between the traits and the functions, genotypes. Thus, the canonical loading reflecting the variance that the observed variables share with the canonical variate, and it can be interpreted in assessing the relative contribution of each variable to each canonical function. The results of canonical discriminate function analysis and cluster analysis appeared to be of complete accordance. The canonical analysis could provide no clear grouping but gave a special idea for genetic variability and most influential characters however, cluster analysis could efficiently describe the characteristics of groups of different genotypes, and both gave a sensible and useful integration of the data. However, more extensive molecular data are needed in order to interpret the best general conclusion about the relationship among the Giza standard varieties and their off-types. The F_2 frequency distribution curves were characterized by a sort of unimodality indicating the continuous type of variation for the studied trait, due to the joint action of polygene. The presence of transgressive segregation in negative direction (lower values) might due depression in later generation which cause a dangerous effect of such off-types. ISSR primers flanked 68 loci in Giza 86 and their off-types with an average 9.71 bands per primer and 61.76 % Polymorphism, While the most important difference revealed SSR primers was a repeat of the band within the genotypes.

CONCLUSIONS

The results from morphological measurements and DNA, ISSR, are complementary factors for each other in studying and identifying the genetic variability and genetic diversity among genotypes and both gave essential information genetic variability in the Egyptian cotton germplasm with providing a useful guide for conserving elite cotton germplasm and eliminate any spontaneous changes from commercial cultivars during the multiplicities stages to maintenance the uniformity and homogeneity of the Egyptian cotton.

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الملخص العربى

تقدير الإختلافات الوراثية التلقائية وعلاقتها بإنخفاض المحصول والجودة فى القطن المصرى

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تعد صفات المحصول وجودة التيلة من الأهداف الأساسية التى يسعى المربى للحفاظ عليها، ويمثل تماثل وتجانس هذه الصفات المعايير الأساسية لتحديد والحكم على نقاوة أصناف القطن والحفاظ على النقاوة الوراثية لأصناف القطن، يوفر الحماية اللازمة ضد إنخفاض الإنتاج والجودة. تم تقييم ومعرفة الإختلافات الوراثية التلقائية بين أصناف القطن الأصلية جيزة ٨٦ وجيزة ٩٤ والطرز المغايرة لكل صنف فى محطة البحوث الزراعية بسخا – معهد بحوث القطن – مركز البحوث الزراعية – مصر خلال مواسم الزراعة ٢٠٢١، ٢٠٢٢ و٢٠٢٣ وأظهرت النتائج التالى:

- وجود إختلافات معنوية بين الأباء الأصلية لكل من الأصناف جيزة ٨٦ وجيزة ٩٤ والطرز المغايرة لكل صنف نتيجة لإختلافات معنوية ظهرت من تفاعل الأباء مع الطرز المغايرة.
- أظهرت الطرز المغايرة تراجع فى معظم صفات المحصول والجودة حيث أظهرت إنخفاض فى قيم معدل الحليج والتصافى مع إنخفاض حاد لصفات طول التيلة مصحوبة بإنخفاض تجانس الطول مع درجة عالية من الخشونة وإنخفاض متانة التيلة جم/تكس.
- كما أظهرت الطرز المغايرة تغيرات فى لون التيلة من اللون الفاتح إلى اللون الكريمى الداكن "البنى".
- أوضحت النتائج أن الإختلافات بين الطرز المغايرة والأباء الأصلية لكل من الصنفين جيزة ٨٦ وجيزة ٩٤ يمكن إرجاعها إلى عاملين، العامل الأول يرجع للإختلاف بين الصنف والطرز المغايرة والعامل الثانى يرجع لمقدرة الصفات فى التعبير عن نفسها أو تطورها.
- أعطت البوادئ الجزيئية لتقنية ISSR حوالى ٦٨ موقع وراثى للصنف جيزة ٨٦ والطرز المغايرة الخاصة به بمتوسط حوالى ٩.٧١ موقع لكل بادئ وكانت نسبة التنوع الوراثى بين الصنف جيزة ٨٦ والطرز المغايرة الخاصة حوالى ٦١.٧٦ %.
- كان الإختلاف الأهم الذى أظهرته البوادئ الجزيئية لتقنية SSR هو عدد مرات تكرار نفس الحزم الجزيئية لكلا الصنفين والطرز المغايرة لهما حيث ظهرت كإختلاف فى تركيز وكمية الحزم الجزيئية على الجل.