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GENETIC BEHAVIOR OF SOME SORGHUM GENOTYPES FOR SOME FUNGI DISEASE RESISTANCE

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ABSTRACT

Three field experiments were carried out at Maryout, Ras-Sudr and EL-Maghara Stations, Desert Research Center, 2003 growing season to study the genetic behavior of nine sorghum genotypes of the M_{10} generation for the severity and incidence of leaf spot disease as well as to compare the fresh and dry forage yield with the parental cultivar (Giza-1). Isozymes (polyphenol oxidase and peroxidase) banding patterns and RAPD-PCR markers were used to genetically identify the genotypes studied for disease resistance. The results obtained revealed that genotypes differ significantly for forage yield at the three locations, however, the genotypes M16, M48, M32 and M43 gave a significant trend for fresh and dry forage yield compared with the parent cultivar (Giza-1). On the other hand, the results showed that genotype M -2 was the most resistant one for fungi disease severity if compare to the parent cultivar (Giza-1) and the other genotypes among the three field experiments. Also, the results revealed that different gene expression levels in the banding patterns when isozymes, which related to disease severity. For RAPD-PCR analysis, six random arbitrary primers were used; the results showed that there are polygenetic relationships between the studied genotypes.

Generally, the biochemical and molecular genetic analysis used in the present study successfully distinguished the fungi disease resistance genotypes of Sorghum bicolor L.

Ke words: Sorghum genotypes, Mutations, Heritability, Isozymes, RAPD- PCR, Fungi disease.

INTRODUCTION

There is shortage in production of annual forage crops which are needed to secure better nutritional requirements of the farm animals in Egypt. The main deficit in livestock requirements is mainly due to the inadequacy of green forage during summer season. *Sorghum bicolor* L. is one of the most important summer forage crops especially in reclaimed and desert areas. Because of its relatively low yield when grown under stresses, a rapid improvement in the yielding could be gained by using the mutation breeding.

Genetic information in sorghum is not extensive as in corn, wheat, or barley, however, genetic studies of interest to the breeders have been made on disease resistance .In this respect, its vulnerable to a number of plant diseases including a few foliar diseases, particularly those caused by pathogenic fungi. Sorghum is attacked with some diseases; the most important of them is *Helminthosporium* leaf blight which caused by the fungus *Bipolaris maydis* (Nisikado & Miyake) Shoemaker, teleomorph. This fungus can cause a major foliar disease in sorghum in Egypt, El- shafey (1970). Although some chemicals are effected in managing plant diseases, the use of chemicals had become a subject to public concern and scrutiny. This is because of the potential harmful effect of chemicals on the environment,

genetic resistance to pesticides. The need for developing non–chemical method to disease management is clearly obvious, Delp (1987) .Therefore, sorghum breeders must be aware of the diseases and to give an attention to them in their breeding programs.

The safest and the most effective approaches for managing plant disease is the use of resistance cultivars. The genetic basis of susceptibility to leaf blight is well known and plants resistant to it are widely available and constitute the first line of defense.

During recent decades genetic investigations were urgently need to understand the genetic behavior of the cultivated crops in relation to ecological stresses, Blum (1979) and Jordan and Miller (1980). This could be achieved by studying some biological genetic fingerprints, i.e. isozyme variations and RAPD-PCR.

The main objective of this investigation was to study the performance of some new mutant lines (M_{10}-generation) as regard to the forage yield and some fungi disease in comparison with local cultivars (Giza-1) under three different environments, to determined the best genotype which could be used as a new line to grown under these environments depending on the genetic information.

MATERIALS AND METHODS

MATERIALS

After nine years from single or individual plant selection during sorghum breeding program by using physical and chemical mutagens. Nine promising genotypes were obtained and classified into three groups; I) induced from physical mutagens (γ rays) ; П) induced from chemical mutagens (E.A.T.) and Ш) induced from compound mutagens representing the mutants (M-2, M-15 , M-16 and M-17), (M- 26 and M- 28) and (M- 32, M-43 and M-48) , respectively . The selected mutants as well as parent cultivar (Giza-1) were used to study their genetic behavior after infected them with Fungi disease. To realize this aim the following studies were achieved.

Field experiments:

Three experiments were carried out at the experimental farms of Desert Research Center at Maryout, El-Maghara and Ras-Sudr stations, 2003 growing season to estimate fresh and dry forage yields of this nine mutants compared with the parent cultivar (Giza- 1) at the M_{10} generation for the severity and incidence of leaf spot disease.

All genotypes were sown at the first week of May 2003 in randomized complete block design with three replications. Three cuts were taken after 55, 105 and 155 days from sowing date from all locations except Ras-Suder, where two cuts were taken, yield of square meter was used to estimate forage yield.

Some chemical and physical analyses of soil and irrigation water of the three experimental stations were carried out, (Table 1).

Table (1): Some chemical and physical analyses of soil and irrigation water of the three experimental stations .

Locations	Types	EC dsm^{-1}	pH	Cations me/L				Anions me/L			$CaCO_3$ %	Textural Class
				Ca^{++}	Mg^{++}	Na^{+}	K^{+}	HCO_3^-	Cl^-	$SO_4^{=}$		
Maryout	Soil (0-30)	4.80	7.7	17.3	9.22	30.65	1.09	3.6	32.00	22.66	24.0	Loamy clay
	Water	3.52	7.5	7.02	8.03	17.33	0.42	9.33	16.44	6.87	-----	
El-Maghara	Soil (0-30)	0.9	7.4	4.00	1.50	3.30	0.15	1.80	5.20	1.95	11.7	Sand
	Water	4.06	8.4	11.4	3.48	24.6	0.69	4.40	32.20	3.57	-----	
Ras-Sudr	Soil (0-30)	7.78	7.7	16.8	10.80	48.3	1.9	11.9	45.80	20.1	42.80	Sandy loam
	Water	6.35	7.6	18.9	5.40	37.4	0.29	1.60	44.56	15.85	-----	

Data analysis:

Data were subjected to proper statistical analysis. according to Snedecor and Cochran (1967) for comparison between means, Duncan's multiple range test was also used (Duncan 1955).

Disease experiment:

Helminthosporium leaf blight caused by the fungus *Helminthosporium maydi* was estimated. The reaction in the field was observed during growth stage and the percentage of infection was estimated in each of the three replicates on ten plants for each mutant line. Also, the score of disease index (severity of infection) was estimated according to Khan and Boyd (1969).

Disease index calculation was done as proposed by Scott and Hollins, (1974):

$$\text{Disease index} = \frac{(0 \times a) + (1 \times b) + (2 \times c) + (3 \times d) + \ldots\ldots}{a + b + c + d + \ldots\ldots}$$

Where a, b, c, d and , are the numbers of plants which fall in the score of infection categories 0, 1, 2, 3 and , respectively.

Genetic analysis:

The studied nine mutants and the parent cultivar (Giza-1) were subjected to the following genetic analysis:-

Isozymes electrophoresis:

Two isozymes i.e., poly-phenol oxidase and peroxidase were extracted from plant samples and polyacrylamide gel electrophoresis was carried out according to the method described by Garkova *et al.* (2000). Poly-phenol oxidase was stained according to Butron and Kirchmann (1997) and peroxidase was stained as the method given by Graham *et al.*, (1964).

Gel analysis:

All gels resulted from isozyme electrophoresis were scanned using Gel Doc-2001 Bio-Rad system. The densitometric scanning of the bands was performed on three direction characters. Each band is recognized by its length, width and intensity. Accordingly, relative amount of each band quantity could be measured and scored.

RAPD-PCR:

DNA was extracted according to El-Fiky *et al.*, (2002). DNA quantification involves the use of UV- spectrophotometers. Spectrophotomtric measurement indicates the amount of Ultraviolet irradiation absorbed by the bases of the nucleic acid Sambrook *et al.*, (1989). Five Operon random primers, Table (2) were applied. PCR reactions were conducted according to Williams *et al.*, (1990). The reaction conditions were optimized and mixtures (25-µl total volumes) were composed of dNTPs (200 µM), MgCl2 (1.5 mM), 1X buffer, primer (0.2µM), DNA (100ng), Promega Taq DNA polymerase (2unit). Negative control was included in which all the ingredients were present except template DNA. Amplification was carried out in a thermocycler (UNOII), Biometra programmed for 95°C for 5 min (one cycle), followed by 94°C for 1 min, 36°C for 1 min and 72°C for 2 min (45 cycles) 72°C for 5 min (one cycle), then 4°C (infinitive). Amplification products (7µl) were mixed with 3µl loading buffer and separated on 1.5% agarose gel and stained with 0.5 µg/ml ethidium bromide, and visualized with ultraviolet light sizes were determined by comparisons with the 1kb DNA ladder marker (Promega Inc and photographed by Gel documentation system (Bio-RAD.,GEL Doc Analyze 2000).

Marker nomenclature:

Each RAPD-PCR marker was named by the primer used and DNA fragment size in base pairs (bp) are shown in (Table 2).

Table (2): Six Operon random primers used and sequences

Primer code	Sequences 5' ----------3'
OPO3	CTG TTG CTA C
OPO4	AAG TCC GCT C
OPO6	CCA CGG GAA G
OPO7	CAG CAC TGA C
OPO10	TCA GAG CGC C
OPO13	GTC AGA GTC C

RESULTS AND DISCUSSIONS

Field studies:

Data presented in Table (3) indicated that genotype mean squares were highly significant for fresh and dry forage yield per feddan in the all cuts, indicating that these genotypes differ in their genetic potential for forage yield. Such variability among different sorghum genotypes in forage yield was also reported by El-Hosary *et al.*, (1995) and Omar (1999).

The mean performances of mutant lines as well as (Giza-1) cultivar for forage yield are presented in Table (4). Data revealed that genotypes were significantly differed for fresh and dry forage yield for each cutting as well as total forage yield at the three locations. The decreased of fresh and dry forage yield for all cuts and total forage yield in Ras-Sudr location is due to the increasing salinity of soil and irrigation water compared with the two other locations (Table -1) because the salinity causes injury and stunting of plants.

The data also show clearly that the average total fresh yield of Maryout location in general varied from 43.196 ton/fed for M-26 to 64.438 ton/fed for M- 32. Meanwhile, the average dry forage yield ranged from 14.876 ton /fed for M-26 to 22.541 ton /fed for M-48. Therefore, the genotypes M- 48 and M-32 were considered out yielded from (Giza-1) cultivar in dry forage yield.

The highest values for fresh and dry forage yield at El-Maghara location were obtained by the genotypes M-16 and M-32 which were out yielded from Giza-1 cultivar.

At Ras-Sudr location fresh and dry forage yield were decreased compared with Maryout and El-Maghara locations due to the effect of salinity. However the genotypes M-32 and M- 43 gave the highest mean values for fresh and dry forage yield out yielded from Giza-1 cultivar.

Heritability (h2) values (Table-4) were generally high for fresh and dry forage yield and cumulated fresh and dry forage yield, indicating that the environments under consideration had slightly effect on the inheritance of such characters at M_{10} generation. High heritability estimates for forage yield indicated that selection based on mean would be successful in improving forage yield. Abo-El-Soad *et al.*, (1994) reported that heritability estimates differed according to the population genetic base, traits and environmental factor

Table (3): **Analysis of variance for fresh and dry forage yield in ten sorghum genotypes (M10 generation) under three different locations at 2003 growing season.**

S.O.V	d.f	Fresh forage yield				Dry forage yield			
		1st cut	2nd cut	3rd cut	Total	1st cut	2nd cut	3rd cut	Total
		Maryout location							
Replication	2	0.916	0.143	0.355	1.545	0.129	0.559	0.051	0.536
Genotypes	9	53.210**	19.672**	7.310**	101.916**	7.849**	3.3410**	0.796**	15.075**
Error	15	0.508	0.439	0.223	0.507	0.308	0.357	0.091	1.003
		El-Maghara location							
Replication	2	1.419	0.531	0.811	0.489	0.447	0. 06	0.034	1.160
Genotypes	9	57.258**	14.462**	4.822**	144.56**	4.055**	2.209**	0.794**	14.066**
Error	15	0.859	0.684	0.277	2.278	0.506	0.292	0.022	0.893
		Ras Sudr location							
Replication	2	0.385	0.283	-	0.767	0.081	0.069	-	0.104
Genotypes	9	3.485**	10.519**	-	23.535**	0.533**	0.605**	-	2.146**
Error	15	0.235	0.286	-	0.629	0.056	0.042	-	0.073

* and ** significantly at 0.05 and 0.01 levels of probability , respectively.

Disease studies:

Data presented in Table (5) show significant differences among genotypes for severity under conditions of the three studied locations, while incidence percentages for the ten sorghum genotypes differed significantly at Ras-Sudr conditions only. M-32 M-43 and M-2 genotypes seemed to be the best lines in regard to disease severity at Maryout, EL-Maghara and Ras-Sudr locations, respectively. It is worthy to note that M-2 exhibited the highest resistance for both disease severity and incidence. Such genotype put under consideration for used at Sinai (El- Maghara and Ras- Sudr) as a promising one. The gain of this selection would be depending on the highest

heritability value exhibited by the two parameters of disease resistance at the two locations.

The coefficient of variability (phenotypic or genotypic) is a relative measure of variations. PCV and GCV percentages, Table (5), were relatively high under Sinai conditions than Maryout location. This confirmed the above heritability values detected for such cases. Disease severity under Ras-Saudr conditions showed no great discrepancy between phenotypic and genotypic coefficient of variability suggesting a small effect of environmental factors

Table (4): Means values of fresh and dry forage yield for ten sorghum genotypes (M_{10} generation) at three different locations.

Genotypes	Fresh forage yield (ton/fed)				Dry forage yield (ton/fed)			
	1st cut	2nd cut	3rd cut	Total	1st cut	2nd cut	3rd cut	Total
Maryout location								
Giza-1	22.094 cd	23.088 bc	6.352 e	51.535 e	7.467 c	8.340 b	2.019 d	17.826 bc
M-2	23.480 c	25.080 a	8.809 c	57.369 c	7.354 c	7.972 bc	2.747 bc	18.074 bc
M-15	23.417 c	23.508 b	6.454 e	53.378 d	7.020 c	8.295 b	2.008 d	17.323 c
M-16	21.687 d	25.803 a	10.583 a	58.073 c	6.781 c	9.336 a	3.410 a	19.526 b
M-17	20.755 d	26.247 a	10.233 ab	57.235 c	7.373 c	9.053 a	3.129 ab	19.555 b
M-48	29.801 a	21.806 c	9.497 bc	61.104 b	10.178 a	9.063 a	3.299 ab	22.541 a
M-26	16.633 e	18.892 a	7.642 d	43.196 f	5.523 d	7.036 d	2.318 cd	14.876 d
M-28	25.593 b	18.808 d	9.517 bc	53.918 d	8.555 b	5.956 e	2.915 ab	17.425 c
M-32	30.625 a	23.372 b	10.442 a	64.438 a	10.777 a	7.876 c	3.160 ab	21.814 a
M-43	26.371 b	22.777 bc	9.277 c	58.425 c	9.049 b	7.062 d	3.004 ab	19.115 bc
h^2	99.05	97.82	97.04	99.5	96.22	90.52	89.74	93.76
El-Maghara location								
Giza-1	21.541 e	18.187 c	6.974 fg	46.702 ef	5.359 cd	5.139 ef	2.469 cd	12.967 de
M-2	24.753 d	20.839 b	8.997 bcd	54.588 cd	5.692 bc	6.658 abc	3.046 ab	15.396 bc
M-15	17.821 f	18.327 c	7.863 ef	44.011 f	4.186 d	5.712 cde	2.813 b	12.712 de
M-16	30.662 ab	22.911 a	9.497 ab	63.070 a	6.877 ab	7.401 a	3.311 a	17.590 a
M-17	23.146 de	16.011 d	6.038 g	45.195 f	5.286 cd	4.613 f	1.813 e	11.712 e
M-48	27.616 c	18.572 c	7.756 ef	53.944 b	7.079 a	6.253 bcd	2.302 d	15.633 bc
M-26	23.590 d	18.033 c	8.068 de	49.691 e	6.339 abc	5.288 def	2.180 d	13.807 cd
M-28	27.581 c	20.578 b	9.126 bc	57.285 bc	7.368 a	6.559 abc	3.175 a	17.103 ab
M-32	29.573 b	22.609 a	10.409a	62.591 a	7.502 a	6.767 ab	3.314 a	17.583 a
M-43	31.510 a	18.572 c	8.319 cde	58.401 b	7.678 a	6.218 bcd	2.635 c	16.528 ab
h^2	98.52	95.48	94.57	98.45	88.91	88.32	97.30	94.03
Ras Sudr location								
Giza-1	10.403 cd	6.908 def	-	17.311 cd	3.342 c	2.167 bcd	-	5.509 cd
M-2	11.565 ab	7.759 cd	-	19.323 b	4.106 ab	2.394 bc	-	6.500 b
M-15	11.186abc	7.348 cde	-	18.534 bc	3.787 bc	2.035 cde	-	5.822 c
M-16	9.595 de	6.455 ef	-	16.050 de	3.405 c	1.810 de	-	5.215 d
M-17	11.376 ab	7.782 cd	-	19.157 b	4.158 ab	2.278 bc	-	6.436 b
M-48	10.767 bc	8.212 c	-	18.979 b	4.047 ab	2.471 b	-	6.518 b
M-26	9.035 e	5.111 g	-	14.146 f	3.530 c	1.670 e	-	5.200 d
M-28	8.989 e	6.297 f	-	15.286 ef	3.422 c	1.816 de	-	5.237 d
M-32	11.557 ab	11.567a	-	23.124 a	4.411 a	2.985 a	-	7.376 a
M-43	11.847 a	10.020b	-	21.867 a	4.435 a	2.929 a	-	7.365 a
h^2	93.68	97.35		97.40	90.49	93.51		96.71

L. values following by the same letter (s) are not different at $p \leq 0.05$ of Duncan's multiple Range F- Test.

The former results of breeding indicate clearly that they were much alined with those obtained from some fungi disease,where the genotypes M-32 and M-48, M-16 and M-32 and M-32 and M-43 for Maryout , El -Maghara and Ras –Sude locations,respecrively,gave the highest forage yields.Also, some of these genotypes exihibit relatively the same trend for fungi disease resistance .

Table (5): Disease severity (DS) and disease incidence (DI) of sorghum genotypes grown in Maryout, EL-Maghara and Ras-Sudr locations, naturally infested with *Helminthosporium* leaf blight.

lines	Maryout		EL-Maghara		Ras- Sudr		Mean	
	DS	DI	DS	DI	DS	DI	DS	DI
Giza -1	1.67	88.67	2.67	26.67	1	10.0	1.78	41.78
M-2	2.67	100.0	1.00	20.0	0.00	0.00	1.22	40.0
M-15	3.00	100.0	1.67	33.33	8.0	93.33	4.22	75.55
M-16	4.67	100.0	1.67	46.67	1.33	23.33	2.56	56.67
M-17	3.33	100.00	1.00	20.00	8.00	80.0	4.11	66.67
M-28	5.0	100.00	2.33	40.00	8.00	40.00	5.11	60.00
M-26	3.33	88.67	1.67	33.33	7.00	40.00	4.00	54.00
M-32	1.33	77.33	6.00	60.00	5.00	20.00	4.11	52.44
M-43	3.00	100.00	0.67	13.33	4.00	53.33	2.56	55.55
M-48	5.33	100.00	5.0	60.00	3.00	10.0	4 44	56.67
Mean	3.33	95.46	2.37	35.33	4.53	37.00	3.41	55.93
F.test	*	NS	**	NS	**	**	NS	NS
LSD 0.05	2.51	-	2.24	-	0.30	23.63	-	-
GCV %	30.22	-	67.30	-	69.04	80.58	-	-
PCV %	54.53	-	88.30	-	69.16	89.32	-	-
H %	30.72	-	58.10	-	99.66	81.38	-	-

G.C.V. = Genotypic Coefficient of variability.
P.C.V. = Phenotypic Coefficient of variability.
H = Heritability broad sense .

Genetic studies:

Isozymes electrophoresis

Two isozyme systems (poly-phenol oxidase and Peroxidase) were used for genetic variation of gene expression under different environments and disease conditions of the concerned ten genotypes for sorghum (nine mutants and Giza-1 cultivar).

1- Poly-phenol oxidase Isozyme:

Electrophoretic patterns of Poly-phenol oxidase isozyme are present in Table (6) and Figure (1). The results revealed that high polymorphism in bands number with percentage (88.8%), Table (8) , were noticed between and within all the genotypes and Giza-1 cultivar. Total nine bands were identified, where one band (number 5) was scored as a common band which expressed in all genotypes. While, the eight remainder bands were polymorphic. The results also revealed that a band number (9) was scored only in Giza-1 cultivar and band number (7) showed in genotype M-28, also band number (8) present only in genotype M-17. Therefore, these bands were considered as positive markers for these genotypes. Moreover, all the genotypes took different behavior of the expression among the effected of disease, except the genotypes M-15 and M-16, which gave the same band numbers (3). The results also indicated that genotype M-2 gave a maximum gene / genes expression, which had number of bands (6). Generally, the rate of gene/genes expression of this isozyme was associated with the fungi

disease; therefore, the genotype M-2 was the most resistant for fungi disease.

Table (6): The presence (+) and absence (-) of bands in isozyme poly-phenol oxidase for ten genotypes of sorghum (nine mutants and Giza-1 cultivar)

Band	Giza -1	M-2	M-15	M-16	M-17	M-28	M-26	M-32	M-43	M-48
1	+	+	+	+	+	+	+	-	+	+
2	-	+	+	+	-	-	-	-	-	+
3	+	+	-	-	+	+	+	+	+	-
4	-	+	-	-	+	+	-	-	-	-
5	+	+	+	+	+	+	+	+	+	+
6	-	+	-	-	-	-	-	-	+	+
7	-	-	-	-	-	+	-	-	-	-
8	-	-	-	-	+	-	-	-	-	-
9	+	-	-	-	-	-	-	-	-	-
Total	4	6	3	3	5	5	3	2	4	4

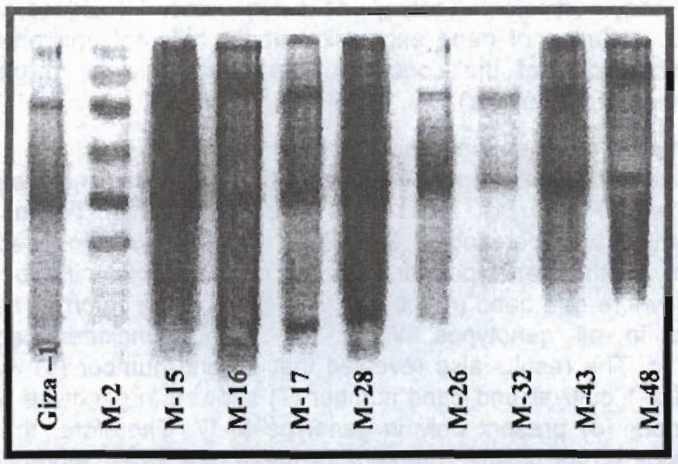

Figure (1): Zymogram of Poly-phenol oxidase banding patterns for ten genotypes of sorghum(nine mutants and Giza-1 cultivar)

2- Peroxidase Isozyme

Banding patterns of peroxidase isozyme for the studied ten genotypes of sorghum illustrated in Table (7) and Figure (2), where these banding showed that this isozyme revealed high polymorphic level with percentage (80.0%), Table (8), and its pattern gave five bands expression among the studied genotypes. The band number (2) was scored as a common band in all genotypes with different in band's intensity between and within them .

The results also indicated that the genotype M-2 gave the maximum gene/genes expression of peroxidase isozyme, meanwhile, genotypes M-16, M-28 and M-32 gave a minimum gene/genes expression of peroxidase isozyme.Thereby,the genotype M-2 was the most resistant ,among the studied genotypes for fungi disease.

Table (7): The presence (+) and absence (-) of bands in isozym peroxidase for ten genotypes of sorghum(nine mutants and Giza-1 cultivar)

Band	Giza -1	M-2	M-15	M-16	M-17	M-28	M-26	M-32	M-43	M-48
1	-	+	-	-	-	-	-	-	+	-
2	+	+	+	+	+	+	+	+	+	+
3	+	+	-	-	-	-	-	-	-	-
4	-	+	+	-	+	-	+	-	+	-
5	+	+	+	-	+	-	+	-	+	+
Total	3	5	3	1	3	1	3	1	4	2

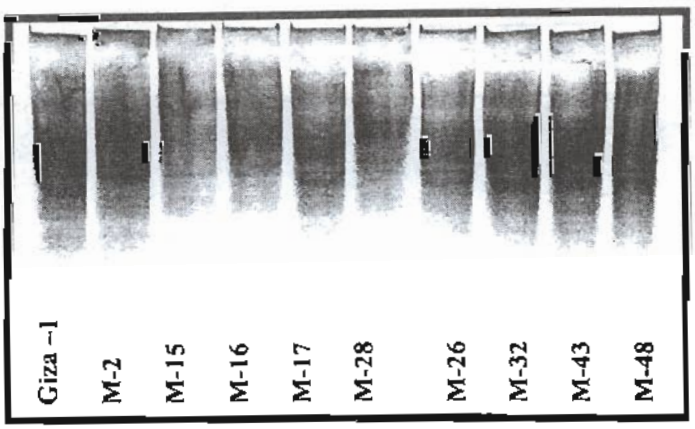

Figure (2):Zymogram of peroxidase banding patterns for ten genotypes of sorghum (nine mutants and Giza-1 cultivar)

Table (8): Number and types of bands as well as the percentage of the total polymorphism generated by two isozymes poly-phenol oxidase and Peroxidase in ten genotypes of sorghum (nine mutants and Giza-1 cultivar)

Isozymes	Monomorphic Bands	Polymorphic		Total bands	Polymorphic
		Unique	non-unique		
Poly-phenol oxidase	1	3	5	9	88.8 %
peroxidase	1	-	4	5	80.0 %

In order to augment the resolution power in this study ,the data of two isozymes electophoretic profiles were combined and applied to the computer to get a dendrogram and similarity matrix as shown in Figure(3) and Table (9) .The dendrogram divided the ten genotypes into two major culasters, the first cluster included all the genotypes and Giza-1 cultivar except genotype M-2 and the second cluster included only genotype M-2 .However, within the first cluster , the genetic distances (Fig. 3) among genotypes varied considerably where the genotypes (M-15,M-16 and M-48) , genotypes (M-28 and M-32) and genotype (M-26 and M-43) were higly related . In addition, the similarity matrix (Table 9) revealed that the highest similarity appeared between genotype M-2 and M-28 (75.0%) while the lowest similarity appeared between genotype M-26 and M-28 (10.1%).

Generally the isozyme system gave good discriminations for all genotypes mutant and Giza-1 cultivar . These results agreed with those obtanied by Henn *et al.*, (1992) who used several isozymes to including poly-phenal oxidase and peroxidase to identify seventeen tomato cultivars.

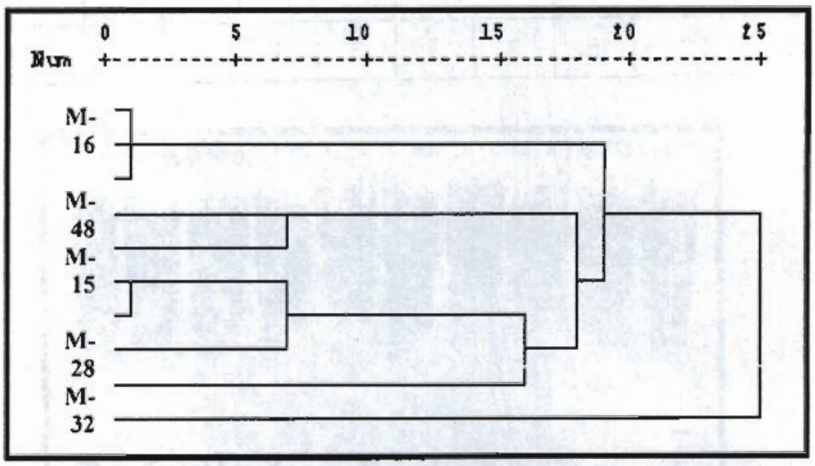

Figure (3) :The genetic distances between the ten genotypes of sorghum ased on isozymes analysis (nine mutants and Giza-1 cultivar)

Table (9) : Similarity matrix between the ten genotypes of sorghum(nine mutants and Giza-1 cultivar) based on combined isozymes analysis

	Giza - 1	M-2	M-15	M-16	M-17	M-28	M-26	M-32	M-43	M-48
Giza -1	00.0									
M-2	17.4	00.0								
M-15	28.9	45.2	00.0							
M-16	31.6	33.0	73.0	00.0						
M-17	28.9	25.1	45.8	22.8	00.0					
M-28	28.9	10.1	12.5	41.1	45.8	00.0				
M-26	57.7	45.2	70.8	41.1	75.0	41.7	00.0			
M-32	52.2	27.3	25.1	44.0	45.2	60.3	60.3	00.0		
M-43	28.9	60.3	45.8	22.8	41.7	16.7	75.0	45.2	00.0	
M-48	28.9	45.2	70.8	73.0	16.7	12.5	41.7	25.1	45.8	00.0

Randomly amplified polymorphic DNA (RAPD)

Fifteen 10- mer random primers were used to differentiate between the ten genotypes of sorghum (nine mutations and Giza-1 cultivar) However, only six primers gave reproducible results and were reported as follows.

Primer OPO 3

The results of primer OPO3 were illustrated in Figure (4) and Table (10).it gave amplification products with all the studied ten genotypes .The molecular sizes of the PCR products ranged from 200 bp to 1130 bp. Four out of total six bands were polymorphic which did not necessarily appear in all genotypes. Two bands of 840 and 400 bp were common to all cultivars, but genotypes M-2 could be distinguished from all other genotypes by the presence of one unique fragment at 200 bp.

Primer OPO 4

The results of primer OPO4 are present in Figure (5) and Table (11). The primer gave amplification products with all genotypes. This primer exhibted a maximum of seven bands with molecular size ranged from 420 bp to 1180 bp. Three common bands were observed in all genotypes at 865,760 and 420 bp. On the other hand, a band number (1) at 1180 bp. was present only in genotype M – 48, therefore, it could be considered as a positive marker of this genotype. While, band number (2) at 1140 bp was present in all genotypes and (Giza – 1) cultivar, except genotype M – 48, it could be considered as a negative marker for this genotype.

Primer OPO 6

The results of primer OPO6 were demonistrated in Figure (6) and Table (12). The results indicated that seven bands as a maximum with molecular sizes ranged from 330 to 1950 bp were exhibited by this primer. However, four common bands were observed in all genotypes at

1500,1100,605 and 330 bp. Meanwhile, only one band was detected in the genotype M – 28 which represents as a positive marker for this genotype.

Primer OPO 7

The results of this primer is present in Figure (7) and Table (13). From the results, it is obvious that the primer gave about nine bands ranged from 325 to 1920 bp. Two bands with molecular sizes of 1530 and 1120 bp were remarked in all studied genotypes and only one band was accompained the genotype M-26 at 665 bp.This band could be taken into consederation as a positive marker for M-26 genotype.

Primer OPO 10

Table (14) and Figure (8) represent the results of primer OPO10. It produced a maximum of eight bands which ranged from 425 to 2500 bp.It was noticed that one band at molecular size 850 bp.was present in all genotypes.However,only two bands were correlated with two genotypes M-26 and M—43 at molecular size 1070 bp.and 1330bp., respectively. Therefore, these two bands could be used as positive markers for these genotypes.

Primer OPO 13

The results of primer 013 are shown in Table (15) and Figure (9). It gave a maximum of eight bands which ranged from 400 to 2450 bp. These was one band at molecular size 840 bp., which was a common band in all studied genotypes, however, only one band was absent with M-43 genotype at molecular size 485 bp., which could be represnts as a negative marker for this genotypes.

The dendogram, based on all RAPD markers developed by all primers of this study (Figure 10) separated the ten genotypes of sorghum into two clusters , genotypes M-2, M-15, M-16, M-17, M-28, M-26, M-32, M-48 and Giza-1 cultivar belong to the same cluster while only genotype M-43 belonge to the other cluster. However, within the first cluster Giza-1, M-28 , M-16 and M-17 genotypes were closely related as well as genotypes M-2 and M-15 . The similarity matrix Table (16) revealed that gentypes M-16 and M-17 were related with a similarity values of 76.2%, while genotypes M-16 and M-43 were quite different with a low similarity of about 1.40% .

RAPD analysis seem to be one of the powerfull tools for detecting polymorphysim and could discriminate between all the ten genotypes . These results agree with Foolad *et al.,* (1993) who found that 63% of the RAPD primers detected one or more polymophic DNA fragment between the studied tomato varities .

From the previous results it could be concluded that the selected nine sorghum genotypes were differed for genetic behavior when they were evaluated from some Fungi disease under different environments .In this respect, the genetic analysis indicated that the genotype M-2 was the most resistant for Fungi disease compared with the parent and other genotypes.So, it could be used as the best genotype in sorghum breeding program for disease resistance. On the other hand we can registed this genotype as a new cultivar to grow under the new reclamid lands .

Tables (10 -15): Survey of polymorphic and monomorphic RAPD bands of the ten genotypes of sorghum (nine mutants and Giza-1 cultivar) using six primers.

Table (10): primer (O-3).

B.No.	Opo3	Giza -1	M-2	M-15	M-16	M-17	M-28	M-26	M-32	M-43	M-48
1	1130	1	1	1	0	0	1	0	0	1	0
2	840	1	1	1	1	1	1	1	1	1	1
3	730	1	0	1	1	1	1	0	1	1	1
4	640	1	0	0	1	1	1	1	1	1	1
5	400	1	1	1	1	1	1	1	1	1	1
6	200	0	[illegible]	0	0	0	0	0	0	0	0
Total		5	4	4	4	4	5	3	4	5	4

Table (11): primer (O-4).

B.No.	Opo4	Giza -1	M-2	M-15	M-16	M-17	M-28	M-26	M-32	M-43	M-48
1	1180	0	0	0	0	0	0	0	0	0	[illegible]
2	1140	1	1	1	1	1	1	1	1	1	[illegible]
3	970	0	0	1	1	0	0	0	0	0	0
4	865	1	1	1	1	1	1	1	1	1	1
5	760	1	1	1	1	1	1	1	1	1	1
6	630	1	1	1	1	1	0	1	1	1	0
7	420	1	1	1	1	1	1	1	1	1	1
Total		5	5	6	6	5	4	5	5	5	4

Table (12): primer (O-6).

B.No.	Opo6	Giza -1	M-2	M-15	M-16	M-17	M-28	M-26	M-32	M-43	M-48
1	1950	1	1	0	1	1	1	1	0	1	1
2	1500	1	1	1	1	1	1	1	1	1	1
3	1100	1	1	1	1	1	1	1	1	1	1
4	680	0	0	0	0	0	[illegible]	0	0	0	0
5	605	1	1	1	1	1	1	1	1	1	1
6	445	1	1	1	1	1	1	1	1	0	0
7	330	1	1	1	1	1	1	1	1	1	1
Total		7	7	5	7	7	8	7	6	6	6

Table (13): primer (O-7).

B.No.	Opo7	Giza -1	M-2	M-15	M-16	M-17	M-28	M-26	M-32	M-43	M-48
1	1920	1	1	1	0	1	1	0	1	1	0
2	1530	1	1	1	1	1	1	1	1	1	1
3	1120	1	1	1	1	1	1	1	1	1	1
4	800	1	0	1	1	1	1	1	1	1	0
5	665	0	0	0	0	0	0	[illegible]	0	0	0
6	645	0	0	0	0	0	1	0	0	1	0
7	600	1	1	1	1	1	0	1	1	0	1
8	500	1	0	0	1	0	1	0	0	0	0
9	325	1	0	0	1	1	1	1	0	0	0
Total		7	4	5	6	6	7	6	5	5	3

Table (14): primer (O-10).

B.No.	Opo10	Giza -1	M-2	M-15	M-16	M-17	M-28	M-26	M-32	M-43	M-48
1	2500	1	0	0	0	0	0	0	0	1	1
2	1740	1	0	0	0	0	0	1	0	1	1
3	1330	0	0	0	0	0	0	0	0	[illegible]	0
4	1070	0	0	0	0	0	0	[illegible]	0	0	0
5	1055	1	1	1	0	0	1	0	1	1	1
6	850	1	1	1	1	1	1	1	1	1	1
7	490	0	1	1	1	1	1	0	1	1	1
8	425	0	1	1	1	1	1	0	0	0	1
Total		4	4	4	3	3	4	3	3	6	6

Table (15): primer (O-13).

B.No.	Opo13	Giza -1	M-2	M-15	M-16	M-17	M-28	M-26	M-32	M-43	M-48
1	2450	1	1	1	1	1	0	0	1	1	0
2	1760	0	0	1	1	0	0	0	0	0	0
3	1300	1	1	1	1	1	0	0	1	1	0
4	1050	0	0	0	0	0	0	0	1	1	0
5	1030	1	1	1	1	0	1	1	1	0	0
6	840	1	1	1	1	1	1	1	1	1	1
7	485	1	1	1	1	1	1	1	1	[illegible]	1
8	400	1	1	0	1	1	1	0	1	0	1
Total		6	6	6	7	5	4	3	7	4	3

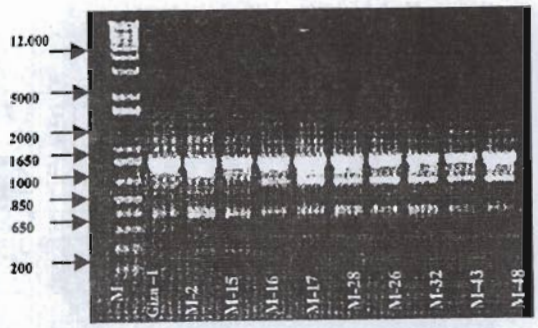

Figure (4) : Primer (O-3)

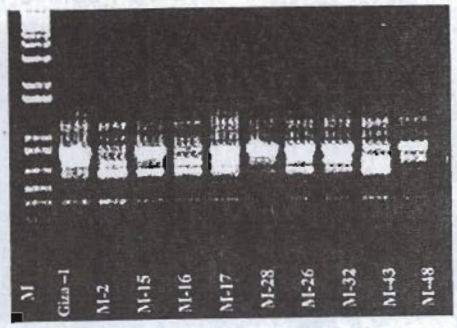

Figure (5) : Primer (O-4)

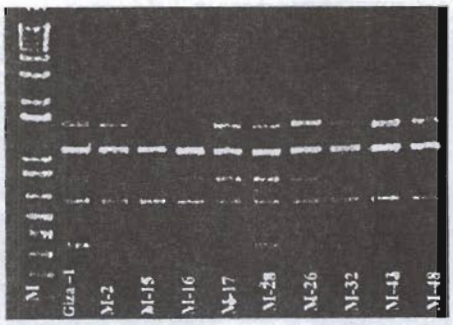

Figure (6) : Primer (O-6)

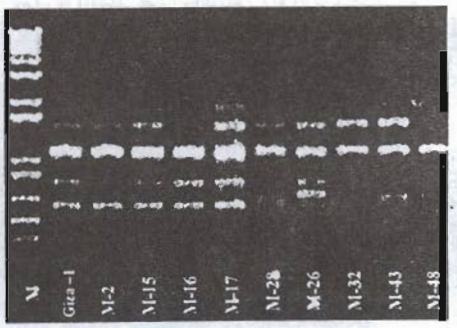

Figure (7) : Primer (O-7)

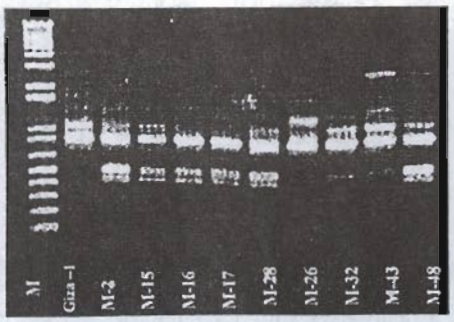

Figure (8) : Primer (O-10)

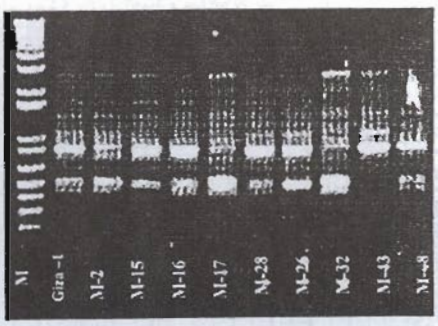

Figure (9) : Primer (O-13)

Figures (4–9) : DNA polymorphism of the ten genotypes of sorghum (nine mutants and Giza-1 cultivar) using RAPD-PCR with primers.

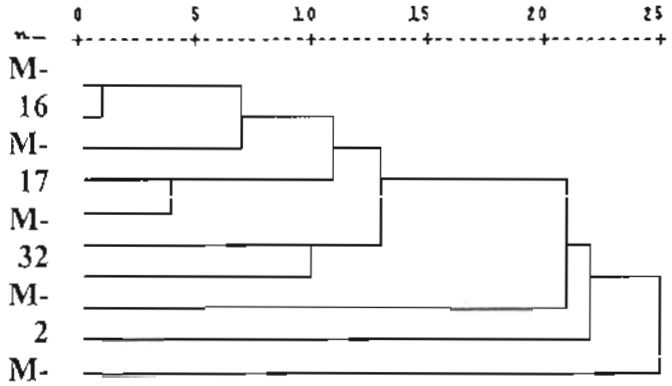

Figure (10) :The genetic distances between the ten genotypes of sorghum (nine mutants and Giza-1 cultivar) based on DNA analysis

Table (16) : Similarity matrix between the ten genotypes of sorghum (nine mutants and Giza-1 cultivar) based combined DNA analysis

DNA	Giza -1	M-2	M-15	M-16	M-17	M-28	M-26	M-32	M-43	M-48
Giza -1	00.0									
M-2	38.7	00.0								
M-15	43.0	70.0	00.0							
M-16	47.6	39.3	56.6	00.0						
M-17	51.6	52.4	57.9	76.2	00.0					
M-28	52.5	45.2	49.9	54.8	57.8	00.0				
M-26	47.5	23.6	29.0	46.9	47.1	16.0	00.0			
M-32	56.1	57.9	63.3	56.6	70.0	49.9	41.0	00.0		
M-43	34.3	14.7	31.7	01.4	27.0	28.9	10.4	44.1	00.0	
M-48	34.7	35.4	29.0	34.8	47.1	28.4	18.3	41.0	22.6	00.0

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السلوك الوراثي لبعض التراكيب الوراثية من السورجم لمقاومة بعض الأمـــراض الفطرية

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تم تنفيذ ثلاث تجارب حقلية في محطات مركز بحوث الصحراء (مريـــوط- السغـــارة - راس سدر)
خلال موسم ٢٠٠٣ وذلك لتقييم تسعة تراكيب وراثية مختلفة من السورجم مع الصنف جيـــزة - ١ لمحصول العلف الأخضر والجاف في الجيل العاشر ودراسة بعض الأمراض الفطرية التي تـــؤثر على إنتاجية محصول العلف حيث زرعت التراكيب في قطاعات كاملـــة العشـــوائية فـــي ثـــلاث مكررات كما تم إجراء الدراسات الوراثية باستخدام التفريد الكهربي لمشابهات الإنزيمات (البولي فينول اوكسيديز والبيرواوكسيديز) وتقنية التكبير العشوائي المتعدد الصـــور للحمـــض النـــووي DNA (RAPD-PCR)لتحديد الاختلافات الوراثيـــة بـــين هـــذه التراكيـــب والصـــنف الأب التجاري(جيزة -١) الناتجة منة .

وقد أظهرت النتائج مايلي:-

١- وجود اختلافات معنوية بين التراكيب الوراثية المختلفة تحت جميع المواقع المدروسة وذلـــك بالنسبة لمحصول العلف والمقاومة للفطريات- وأيضا أظهرت هذه التراكيب اختلافات واضحة في سلوكها الوراثي بالنسبة للتعبير الجينى لمشابهات الإنزيمات المرتبطة بالمقاومة للفطريـــات وأيضا المرتبطة بالاجهادات البيئية

٢-أظهرت التراكيب الوراثية أرقام M - ١٦، M -٤٣، M -٤٨ أعلى قـــيم لمحصـــول العلـــف الأخضر والجاف تحت جميع المواقع المدروسة وكذلك تحملها للاصابة الفطرية.

٣- أظهر التركيب الوراثي رقم M-٢ درجة عالية من المقاومة للفطر مقارنة بالأب والتراكيـــب الوراثية الأخرى وتأكد ذلك من خلال الدراسات البيوكيميائية الوراثية بالتفريد الكهربي للتعبيـــر الجينى لأنزيمي(البولي فينول اوكسيديز - البيرواوكسيديز) حيث تميز هذا التركيـــب بتعبيـــر جينى خاص يمكن اعتباره دلائل وراثية ايجابية لمقاومة الفطريات وتحمل الاجهادات .كما كانت درجة التوريث عالية لمحصول العلف في كل المواقع مما يشير إلى قلة تأثير البيئة على توريث هذه الصفات في الجيل العاشر.

٤- أوضحت النتائج الجزيئية الوراثية باستخدام تقنية التكبير العشوائي المتعدد الصـــور للحمـــض النووي DNA (RAPD-PCR) باستخدام ستة بادئات عشوائية وجود تباينـــات بـــين هـــذه التراكيب الوراثية لهذه البادئات بينما تميزت بعض التراكيب M -٢بالحزمةرقم ٦ للبـــادئ 3-OPO- والتركيبM -٤٣ بالحزمة رقم ٣مع البـــادئ10- OPO والحزمـــة رقـــم ٧مـــع البادئ13-OPO والتركيبM -٤٨ بالحزم أرقـــام ١-٢مـــع البـــادئ4- OPO . وهـــذه الكشافات تعتبر أدلة مساعدة للانتخاب لمقاومة الفطريات وأيضا الاجهادات البيئية المختلفة.