GENETIC VARIABILITY AT THE *b*-MATING TYPE LOCUS IN *Ustilago maydis* IN EGYPT AND ITS MOLECULAR IDENTIFICATION

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Abstract: This present investigation was carried out to study the variation at the *b* mating type in the basidiomycete fungus *Ustilago maydis*, the causal agent of corn smut disease. Different isolates were collected from: Assiut, Sohag, Menia, Sharqiya, Daqahlia,Giza and Kalubia governorates in Egypt.

Eight different *b* mating types were identified among the samples with approximately equal frequencies. The b_1 , b_3 and b_8 alleles were the most frequent, whereas the alleles b_4 and b_6 were the least frequent. The allele b_4 was absent in Sohag samples whereas the allele b_6 was absent in Assiut, Menia and Lower Egypt samples.

The gene diversity G_{st} (population differentiation) for *b* mating types within and between subpopulation were calculated. The total diversity over all populations was 85.86 and the average gene diversity within populations was 82.79 and the gene diversity among population was 3.07 indicating that the majority of gene diversity was occurring in small spatial scale. Low population differentiation was indicated by the low G_{ST} values among populations, suggesting corresponding high degree of gene flow in these population. Ten *U. maydis* samples collected from four geographic regions (Assiut, Sohag, Menia and Lower Egypt) and belonging to different *b* mating types had been selected for RAPD analysis**.** Such analysis generated 40 discrete DNA bands ranging from 150-2500 bp in size. Twenty seven of which were polymorphic (67.50), with an average of 8 bands per primer. One putative a_2 mating type specific band was generated with primer OPO-12 of 2400 bp in size.

Results indicated that no relationship between the RAPD profile and geographic sites where isolates were collected from.

Key words: *Ustilago maydis*, genetic variability, *b* mating type locus.

Introduction

Maize (*Zea mays* L.) is a major cereal crop in Egypt which is subjected to a relatively large number of pathogenic fungi. The smut fungi represent the most harmful fungi that cause severe losses in crop productivity of maize in the field as well as in storage (Asran, 1993). *Ustilago maydis* is a member of the smut fungi which induces tumor formation in maize, and requires passage through the plant in order to complete its life cycle. It can exist as a unicellular yeast-like haploid form or as a filamentous dikaryotic form. This dimorphic transition entails both a

switch from a nonpathogenic to a pathogenic state as well as a switch from a saprophytic to parasitic form (Banuett, 1995) .

Three distinguishable forms in the life cycle of *Ustilago maydis* could be recognized; (a) unicellular and uninucleate haplodid form (sporidium) which is saprophytic (can grow on non-living matter); (b)dikaryotic filamentous form, which is parasitic (need the plant for growth); and (c) a diploid form (teliospore), which is only formed within the tumors. These morphological transitions are governed by two mating type loci, *a* and *b*, both of which must be heterozygous for compatibility of mating haploids (Banuett, 1991; Bölker, 2001 and Casselton, 2002). The *a* locus has two alleles, a_1 and a_2 and controls the signaling and cell fusion events (Banuett, 1991). The *b* locus which acts also as a genetic "master switch" and controls the transition from a harmless saprophytic yeast-like phase to filamentous form that invades the plant is represented by up to 25 different alleles (Puhalla, 1970; Barrett, *et al*., 1993; Gillissen, *et al*., 1992 and Rowell, 1955). Each *b* allele is made up of two divergently transcribed genes, termed *b* East (*bE*) and *b* West (*bW*) whose products are acting in combination to regulate additional genes involved in filamentous growth and pathogenicity (Banuett, 1991; Barrett, *et al*., 1993; Giasson and Kronstad, 1995; and Gillissen *et al*., 1992).

Although the variation at the *b* locus is of direct importance to the population genetic structure and pathogenicity of *U. maydis*, little is known about the evolutionary processes maintaining its diversity. Recent molecular analyses of fungal and mating compatibility type genes demonstrated that different mating types are highly divergent in sequence and have likely been maintained in population for long periods (Clark and Kao 1991 ; Gillissen, *et al*., 1992 ; Kronstad and Leong , 1990 ; and Richman,*et al*.,1996).

The present investigation was performed to determine, for the first time in Egypt, levels of variation, geographic distribution and frequency distributions of alleles at the *b* mating type locus.

Materials and Methods

I. Collecting of *U. maydis* isolates:

Smut galls were collected from diseased maize plants in different locations: three isolates from each of Sharkiya, Dakahlyia, Kalubyia, Giza governorates**;** 6 isloates from Menia**;** 12 isloates from Assiut and 9 form Sohag governorates of Arab Republic of Egypt.

Galls tissue was ground in small morter and debris were removed. Then Teliospores were suspended in

1.5 percent Copper sulfate ($CuSO_4$) solution overnight to remove contaminants and vegetative cells according to Holliday (1974).

II. Isolating and mating haploid sporidial stage:

Mature teliospores (diploid spores) were plated at low densities on nutrient agar medium. They were incubated for 36 to 48 h at 25°C to undergo meiosis and produce probasidia and sporidia. Individual sporidia, representing meiotic segregants, were recovered after streaking the microcolonies.

III. Determination of *b* mating types by plate matings :

Sixty single haploids derived from two teliospores obtained from two separate isolates, were used to determine the tester strains. The genotype of the first sporidia was arbitrarily considered a_1b_1. Mating types were identified in two screens, the first identified the *a* mating type of each sporidium by pairing it with the "testers" arbitrarily assigned $a_1\ b_1$, $a_1\ b_2$, $a_2\ b_1$, and $a_2\ b_2$ mating types. Identical *b* mating types were identified as noncompatible by plate mating, onto rich agar medium, when the fuzz reaction indicated that filamentous growth did not occur (Holliday, 1974 and Zambino, *et al*., 1997) as demonstrated in Fig. 1. The second screen identified the *b* mating types other than b_1 and b_2 using previous testers. To ensure the recovery of all b mating types from each collected gall, colonies representing 50-60 haploid sporidia were isolated from each gall.

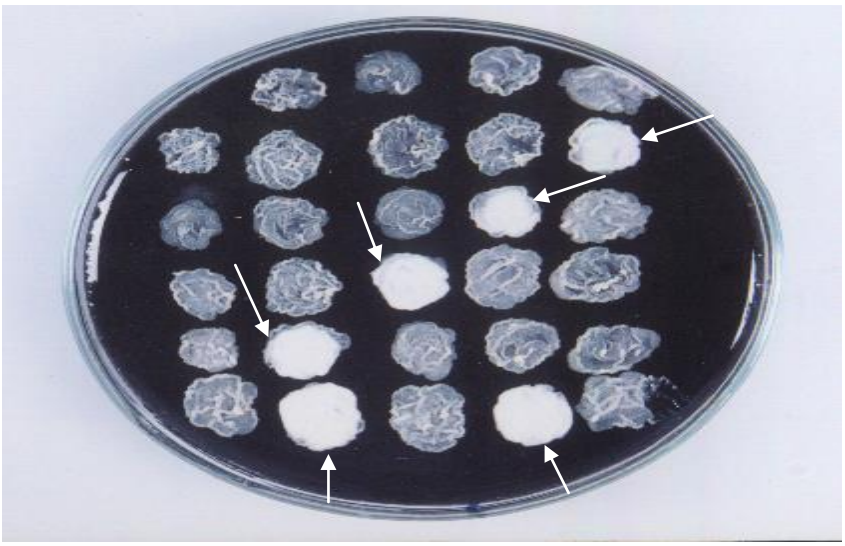


Fig.(1): Mating reaction in *U.maydis*: Formation of hyphae (Fuz^+) marked with white arrows; no hyphae formation (Fuz^-) unmarked.

IV. Media composition

Yeast extract peptone dextrose (YEPD) medium was composed of 2% dextrose, 2% peptone, 1% yeast extract, distilled water up to 100 ml and 2% agar was added for medium solidification.

Nutrient agar medium was prepared using 0.3 % beef extract, 0.5% peptone and 1.5% agar and 100 ml distilled water.

Mating medium was composed of 4 % dextrose , 4.0 % peptone, 2.0 % yeast extract, 1.0 % activated charcoal and 2.7 % agar and 100 ml distilled water (Day and Anagnostakis 1971 and Holliday, 1974).

V. Data analysis:

The Mantel test was used for testing the null hypothesis of equal frequencies among *b* mating types (Lawrence *et al.*, 1994 and Mantel 1974).

VI. RAPD-PCR-analysis :

RAPD assays were based on the polymerase chain reaction (PCR) amplification of random sites spread allover the genomic DNA of ten samples, representing different *a* and *b* mating types. Such assays were conducted using five arbitrary decamer primers (Operon Technologies inc. Alameda CA, USA) the nucleotides of which are given in Table 1.

Table(1): Nucleotide sequences of the five primers used in the RAPD analyses.

Primer code No.	Nucleotide sequences 5' to 3'
OPB-10	5'-CTGCTGGGAC-3'
OPO-06	5'-CCACGGGAAG-3'
OPO-12	5'-CAGTGCTGTG-3'
OPZ-07	5'-CCAGGAGGAC-3'
OPZ-08	5'-GGGTGGGTAA-3'

DNA isolation.:

A single colony representing a haploid of each sample was picked and resuspended in 150 ml of distilled H_2O. The suspension was boiled for 5 min, and the supernatant was collected after spinning for 2 min. in a microcentrifuge. The DNA concentration of boiled extracts was determined with a DNA Dipstick kit (Invitrogen ، San Diego, Calif., Phenol-chloroform-extracted). DNA was isolated as described by Silhavy *et al*., (1984) .

PCR conditions:

PCR amplifications were performed following the procedure of Williams et al ., 1990 and 1993). The reaction was carried out in a 50µL

containing 10 ng of genomic DNA, 10 µM of random primer, 10X Taq polymerase buffer, 2 mM dNTPs, 50 mM $MgCl_2$. Thermal cycle was programmed as follows: an initial denaturation step at 94°C for 5 min followed by 35 cycles of 94°C denaturation for 45 s, annealing at 36°C for 30 s and a final extension at 72 °C for 7 min. The samples were cooled at 4°C. The amplified DNA fragments were separated on 2% agarose gel and stained with ethidium bromide. The amplified pattern was visualized using a UV light. One kbp DNA ladder; 100 bp ladder and Lamda phage DNA digested with Hind III were used as standard DNA markers.

RAPD Data Analysis:

For each primer, a matrix of all bands detected in different fungal isolates was generated using "1" when the band was present and "0" when the band was absent. Statistical analysis was performed using Numerical Taxonomy and Multivariate Analysis System (NTSYS-PC, Rohlf, 1993) Version 2.01. Nei's coefficient was used for genetic similarity (Nei and Li, 1979). Cluster analysis was carried out using the unweighted pair group mean average method (UPGMA) (Sneath and Sokal, 1973).

Results And Discussion

Determination of mating type:

The mating system of *U.maydis* is controlled by two loci, *a* and *b* which must carry different *a* and *b* alleles to be completely compatible and allow the completion of the sexual cycle on corn. Genotyping of the first screen was performed and the genotypes of the five initial isolates are given in Table 2. *Ustilago maydis* mating types were surveyed at seven governorates in Egypt to determine levels of variation, geographic distribution and allele frequency at the *b* mating type locus using plate mating methods. A total of 39 isolates were recovered from different locations in Egypt. Seventy-eight haploids were determined in all isolates.

Table(2): Mating reaction of initial haploid isolates.

Isolates	1(a_1b_1)*	2(a_2b_2)	3(a_1b_2)	4(a_2b_1)	Genotype
Assiut1-1	-	+	-	-	a_1b_1*
Assiut1-2	+	-	-	-	a_2b_2
Assiut1-3	-	-	-	+	a_1b_2
Assiut1-4	-	-	+	-	a_2b_1
Assiut2-1	+	-	+	-	a_2b_3

+Formation of hyphae (Fuz^+); - no hyphae (Fuz^-) *Arbitrarily assigned a_1b_1

Table(3): *U . maydis b* matig types obtained from different isolates and

locations in Egypt.

Isolate Serial no	Location	Season	*b* mating types							
			*b*1	*b*2	*b*3	*b*4	*b*5	*b*6	*b*7	*b*8
1	Assiut	2001	+	+						
2	Assiut	2001	+		+					
3	Assiut	2001		+		+				
4	Assiut	2001				+			+	
5	Assiut	2001	+				+			
6	Assiut	2002					+			+
7	Assiut	2002	+		+					
8	Assiut	2002							+	+
9	Assiut	2002			+		+			
10	Assiut	2002							+	+
11	Assiut	2002	+			+				
12	Assiut	2002			+					+
13	Sohag	2002	+		+					
14	Sohag	2002			+					+
15	Sohag	2002		+			+			
16	Sohag	2002							+	+
17	Sohag	2002		+					+	
18	Sohag	2002	+					+		
19	Sohag	2002		+				+		
20	Sohag	2002		+						+
21	Sohag	2002			+			+		
22	Menia	2002	+		+					
23	Menia	2002				+	+			
24	Menia	2002					+		+	
25	Menia	2002	+	+						
26	Menia	2002	+		+					
27	Menia	2002				+	+			
28	Giza	2002				+	+			
29	Giza	2002					+			+
30	Giza	2002		+					+	
31	Kalubyia	2002			+					+
32	Kalubyia	2002			+				+	
33	Kalubyia	2002			+				+	
34	Sharkyia	2002		+					+	
35	Sharkyia	2002			+					+
36	Sharkyia	2002	+		+					
37	Dakahlyia	2002							+	+
38	Dakahlyia	2002	+		+					
39	Dakahlyia	2002					+			+

For the other isolates, locations and *b* mating types determined are presented in Table (3). Of the 24 haploids recovered from Assiut and Lower Egypt governorates (Giza, Kalubia, Scharkia, Dakahlia), seven different mating types were identified. Samples from Sohag governorate yielded seven different mating types out of 18 haploids, whereas those from Menia governorate yielded six different mating types from 12 haploids. Eight different *b* mating types were identified all over the samples.

Allele frequencies of *b* mating type :

The frequencies of *b* mating type alleles in isolates collected from different locations in Egypt and the overall population frequency are presented in Table 4 and illustrated in Fig 2.

Averaged over locations, the observed frequencies of the eight different *b* mating types ranged from 0.04 to 0.15. No allele was recovered at a frequency greater than 0.25 at any location. The Mantel test showed that overall isolates, the eight mating types occurred approximately at equal frequencies throughout the entire population and in all subpopulation samples (Table 5). The b_1, b_3 and b_8 alleles were the most frequent, whereas the alleles b_4 and b_6 were the least frequent. The allele b_4 was absent in Sohag samples, whereas the allele b_6 was absent in Assiut, Menia and Lower Egypt samples.

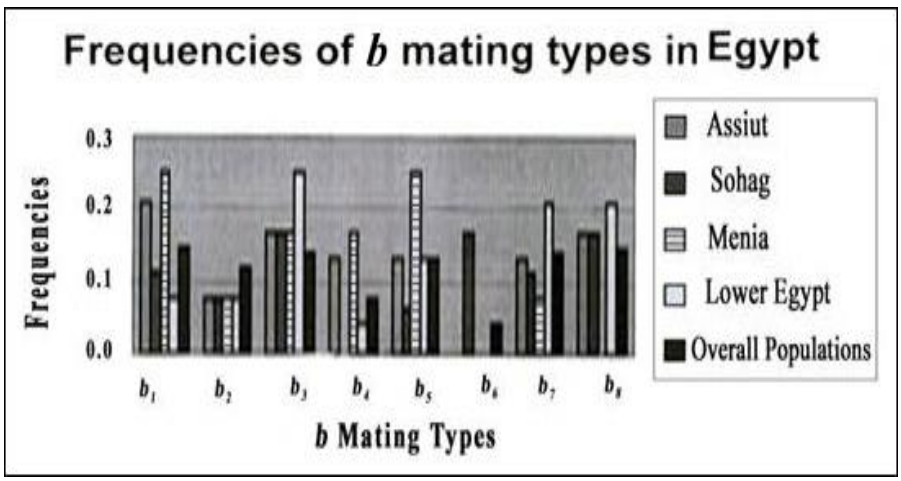


Fig.(2): Frequencies of *b* mating types in Egypt.

Table(4): Allele frequencies of *b* mating type locus in field populations of *Ustilago maydis* collected from Assiut, Sohag, Menia and Lower Egypt during the 2001 and 2002 maize growing seasons.

alleles	Assiut	Sohag	Menia	Lower Egypt	All over populations
b_1	0.21	0.11	0.25	0.08	0.15
b_2	0.08	0.22	0.08	0.08	0.12
b_3	0.17	0.17	0.17	0.25	0.14
b_4	0.13	0.00	0.17	0.04	0.08
b_5	0.13	0.06	0.25	0.13	0.13
b_6	0.00	0.17	0.00	0.00	0.04
b_7	0.13	0.11	0.08	0.21	0.14
b_8	0.17	0.17	0.00	0.21	0.15

Table(5): Occurrence of *b* mating type alleles of *U.maydis* sampled in different locations.

b alleles	Assiut	Sohag	Menia	Lower Egypt	Overall populations
b_1	5	2	3	2	12
b_2	2	4	1	2	9
b_3	4	3	2	6	15
b_4	3	0	2	1	6
b_5	3	1	3	3	10
b_6	0	3	0	0	3
b_7	3	2	1	5	11
b_8	4	3	0	5	12
Total	24	18	12	24	78
Hs	84.72	83.95	80.56	82.64	85.86
Mantel test $\chi 2$	2	2.66	0.67	7.6	
d.f.	6	6	5	6	7
Critical value	10.6	10.6	9.24	10.6	12

Non significant with the Mantel value less than the critical chi square value.

These results are in agreement with Doerder *et al.*, (1995) observations for *Tetrahymena thermophila* and Zambino *et al.*, (1997) on *U. maydis* field isolates collected from four locations of Minnesota. The frequency-dependent sex-ratio theory predicts equal frequencies of fixed multiple mating types. A rare mating type should increase in frequency because any cell with that type would have an increased probability of finding a compatible conjugal partner.

Gene diversity within and among populations:

The gene diversity of each population (H_s, heterozygosity) was high with values ranging from 80.56 % for Menia samples to 84.72 % for Assiut samples (Table 5).

The total diversity over all populations was 85.86. The average gene diversity within populations was 82.79 and the gene diversity among population was 3.07 indicating that the majority of gene diversity was occurring in small spatial scale. A large amount of genetic diversity distributed over a small spatial scale suggests the possibility of rapid adaptation by pathogen to changing environment (Watson, 1981). Such a high degree of genetic similarity among populations collected from widely separated geographic regions suggests the occurrence of significant long distance dispersal and gene flow. Gene flow over long distance poses a threat to deployment of resistance genes tailored to total pathogen populations, because immigrants may possess virulence genes that can overcome resistance in local host cultivars. New virulence genes also might be incorporated into local pathogen populations through recombination (Braiser, 1988).

The pairwise coefficient of differentiation (G_{st}) estimate over all populations was 0.036 which corresponds to a gene flow value (Nm) of 13.39 (Table 6). The pairwise G_{st} estimate between subpopulations ranged from 0.011 to 0.048. The lowest pairwise G_{st} estimates and the highest pairwise values of Nm were found in comparisons for the Lower Egypt samples with Assiut samples while the highest pairwise G_{st} values and the lowest value of Nm were found in comparisons of the Sohag samples with Menia samples. Most of the diversity at the *b* mating type was represented within each of the local sample, while little additional diversity was attributed to differences between population. Low population differentiation was indicated by the low G_{st} values among populations, suggesting corresponding high degree of gene flow in these populations.

Few studies have examined the genetic variability in the Ustilaginales (Martinez-Espinoza *et*

al., 2002) and little is known about the population mating behavior of *U. maydis*. A high degree of variability has been found at the *b* locus. The N-terminal regions of the bE and bW- encoded proteins that are highly variable (Barnes *et al*., 2004). At this locus, the requirement of heterozygosity for mating and pathogenicity is hypothesized to act as a strong selective force to maintain variability. Zambino *et al*., (1997) concluded that a high level of variability might be maintained at this locus even in local populations.

Table(6): Pairwise comparisons of population differentiation (G_{ST}, above the diagonal) and gene flow (*Nm*, below the diagonal), in *U.maydis* field populations from Egypt.

	Assiut	Sohag	Menia	lower Egypt
Assiut	….	0.022	0.021	0.011
Sohag	22.43	…	0.048	0.019
Menia	23.31	9.91	…	0.036
lower Egypt	44.95	25.82	13.39	…

Meanwhile, Garton *et al*., (2001) and Valverde *et al*., 2000 observed no correlation between genetic and geographic distance for *U. maydis* populations.

Gene flow was found to be not high between widely separated geographic distances which suggests that much gene flow occurred as smut followed maize through domestication and host range expansion (May *et al.*, 1997). Selection on new *b* types occurred largely in the past when population sizes of host and pathogen were smaller.

Barnes *et al.*, (2004) reported that the mean of inbreeding coefficients (F_{is}) values estimated for two populations from North America and Uruguay were statistically not different from zero suggesting that teliospores infrequently acted as single infection units. The genetic differentiation between populations was high (F_{st} = 0.25).

RAPD analysis:

To investigate the characterization and the degree of genetic diversity between strains, ten *Usilago maydis* samples were selected from the entire panel of isolates. They had been selected from different geographic sites and belonged to different *b* mating types (Table 7). Ten mer oligonuclotide primers were used to generate RAPD profiles (Figures 3 A-E).

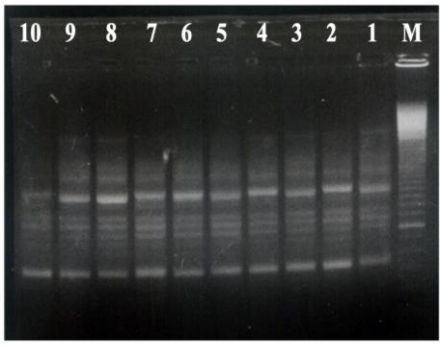
Table(7): Samples of *U.maydis* analyzed in RAPD analysis.

Strains serrial No	Location	Genotype
1	Giza	a_2 b_1
2	Assiut	a_2 b_2
3	Dakahlyia	a_2 b_3
4	Sharkyia	a_1 b_4
5	Assiut	a_1 b_5
6	Sohag	a_1 b_6
7	Kalubyia	a_2 b_7
8	Assiut	a_1 b_7
9	Sharkyia	a_1 b_8
10	Menia	a_2 b_8

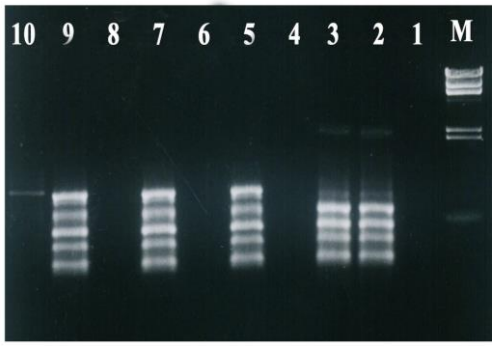
The number of fragments amplified per primer varied between 6 to 9 and had a size ranging from 150 to 2500 bp. A total of 40 DNA bands were amplified at a rate of 8 bands per primer, out of which 25 (67.50 %) were polymorphic. Thirteen bands were equally present in all studied isolates. Primer OPO-10 and OPO-12 generated the largest number (9) of fragments while primer OPZ-08 generated the lowest number (6) of fragments Table 8. Primer OPZ-08 exhibited great discriminatory power in differentiating samples of *U. maydis*.

Table(8): Analysis of the polymorphism obtained with random primers among various *U. maydis* samples.

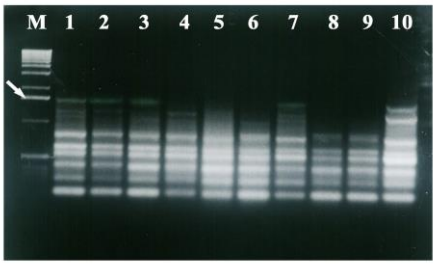
Primer	Total no. of bands (a)	No. of polymorphic bands(b)	Polymorphism% = (b/a)*100
OPB-10	9	2	22.22
OPO-06	8	8	100.00
OPO-12	9	4	44.44
OPZ-07	8	8	100.00
OPZ-08	6	5	83.33
Total	40	27	67.50



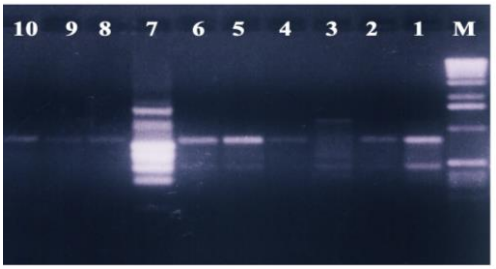
(A)



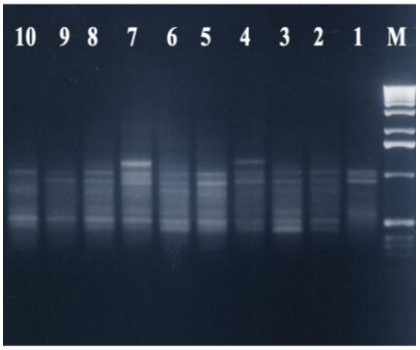
(B**)**



(C**)**



(D**)**



(E**)**

(A) Primer OPB-10 with (M) Molecular weight standard (100bp DNA ladder).
(B)Primer OPO-06 with (M) Molecular weight standard (lambda DNA Hind I Marker).
(C)Primer OPO-12 with (M) Molecular weight standard (1 kb ladder).
(D) Primer OPZ-07 with (M) Molecular weight standard (1 kb ladder).
(E)Primer OPZ-08 with (M) Molecular weight standard (1 kb ladder).

Fig.(3): RAPD fingerprinting profile of a collection of 10 samples of *U. maydis* obtained by RAPD-PCR using different primers using isolates from: (1) Giza (a_2b_1), (2) Assiut (a_2b_2), (3) Dakahlyia (a_2b_3), (4) Kalubyia (a_1b_4), (5) Assiut (a_1b_5), (6) Sohag (a_1 b_6), (7) Kalubyia (a_2 b_7), (8) Assiut (a_1b_7), (9) Sharkyia (a_1b_8), (10) Minea (a_2 b_8) governorates (their mating type).

Remarkably, Primer OPO-12 generated one putative a_2 mating type specific band with molecular size of 2400 bp (marked with a white arrow). This unique specific band could be used to distinguish a_2 mating type from a_2 among all the tested samples. This method is simple and could be used in screening *U. maydis* spores and is in accordance with Tanaka, *et al*., (2004), who screened molecular markers linked to the mating factors using the randomly amplified polymorphic DNA (RAPD) method, to develop the mating type identification procedure in *Lentinula edodes*. They discovered four markers for the *A* factor and two markers for the *B* factor. Two RAPDs perfectly segregated with each mating factor among detected 72 basidiospore strains.

Genetic similarity matrix and cluster analysis:

Based on the calculated genetic distance presented in Table 9, an estimation of the relationship with different strains showed that the smallest genetic distance was observed between sample 5 and 9 (0.043) while the highest genetic distance was found between sample 7 and 10 (0.358).

Based on genetic dissimilarity and the dendrogram (Fig.4) for *U. maydis* samples were regrouped in four distant culsters. The first group included sample 5 which was very closely related to 9 .The second group assembled samples 6, 8, 1, 4 and 10 ,with stronger relationship of isolate 6 with isolate 8 and between strains 1 and 4. The third group included sample 2 and 3 and the sample 7 formed alone the fourth group, which is distinct from all other samples.

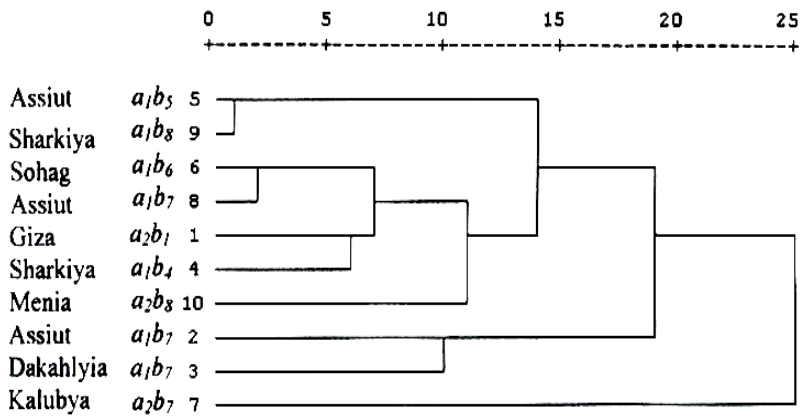


Fig.(4): UPGMA dendrogram of the genetic relationships between different *U. maydis* samples based on RAPD data analysis.

The panel of *U. maydis* samples that had been used included three samples collected from Assiut governorate, two from Kalubyia governorate and one collected from each of Sohag, Menia, Giza, Dakahlyia and Sharkyia governorates. The three samples collected from Assiut governorate, each of which existed in different group clusters, also the two strains collected from Kalubyia governorate existed in two different group clusters, while the two isolates that are very closely related (5 and 9) one of which was collected from Lower Egypt and the other was collected from Upper Egypt. This indicates no relationship between the RAPD profile and geographic origin sites that isolates were collected from. Such results are in agreement with those of Rio and Bamber, (2002), Khalil *et al*., (2003) and Belabid *et al.,* (2004) who reported that when a genetic fingerprint based on a random sample of the genome was compared to a basic suite of geographic variables, the relationship of the two was found to be very poor and not at all predictive. But this does not exclude the possibility that the geographic parameters used might predict some specific genetic quality or that some untested ecogeographic parameter may exist and predicts general genetic diversity.

Table(9): Genetic distance between *U. maydis* samples based on RAPD data.

Isolates	1	2	3	4	5	6	7	8	9	10
1	0.00									
2	0.292	0.00								
3	0.216	0.127	0.00							
4	0.122	0.289	0.333	0.00						
5	0.200	0.184	0.192	0.238	0.00					
6	0.143	0.217	0.265	0.179	0.163	0.00				
7	0.259	0.276	0.180	0.333	0.200	0.308	0.00			
8	0.122	0.244	0.250	0.105	0.190	0.077	0.333	0.00		
9	0.200	0.182	0.192	0.190	0.043	0.209	0.236	0.143	0.00	
10	0.163	0.234	0.320	0.200	0.273	0.171	0.358	0.200	0.280	0.00

Few studies have examined genetic variability in the Ustilaginales (Martinez-Espinoza, *et al.*, 2002) and Kinscherf and Leong (1988) found considerable chromosome length polymorphisms

among various strains of *Ustilago maydis*. Among field and laboratory isolates, none shared exactly the same karyotype. However, the majority followed a similar general pattern. The highest degree of variability was found in laboratory strains, probably due to chromosomal rearrangements, although these strains retained their ability to mate and produce galls in the plant. A high degree of polymorphism was also found at the chromosome ends when telomeric sequences were used as probes (Sanchez-Alonso *et al.*, 1996). Telomere associated sequences of standard laboratory strain (FB2) and wild isolate were studied in detail, and differences between both strains were evident (Sanchez-Alonso and Guzman, 1998) . High level of diversity was also found by RFLP with a set of 23 different probes when *Ustilago maydis* isolates from five different locations in Mexico were analyzed (Valverde *et al*., 2000).

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الاختلافات الوراثية في موقع الطراز التزاوجى ب في فطر يوستيلاجو مايدز في مصر وتمييزه على المستوى الجزيئ

فتحي محمد مصطفى صالح؛ محمد محمود حسيب الدفراوي؛ رأفت فؤاد عبده وأحمد فتحي محمد

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تتناول الدراسة الحالية الاختلافات في موقع الطراز التزاوجى *b* للفطرالبازيدى يوستيلاجو مايدزالمسبب لمرض التفحم العادي في الذرة لعزلات مختلفة من محافظات مختلفه فى مصر.

تم جمع تسعه وثلاثون عينه تحتوى على الجراثيم الثنائية (الجراثيم التيليتيه) من سبع محافظات هي أسيوط وسوهاج والمنيا والجيزه والقليوبية و الشرقية والدقهلية. عزل منها الطور الأحادي للفطر وقد تم تحديد نوع الطراز التزاوجى لثمانية وسبعون من الجراثيم الأحاديه المعزولة. وتم حساب التكرارالأليلي لموقع الطراز التزاوجى *b* في سبعة مواقع جغرافية مختلفة هي أسيوط وسوهاج والمنيا والوجه البحرى (ويضم محافظات الشرقية، الدقهلية، الجيزة والقليوبية) ومنه أمكن حساب التنوع الجينى لموقع الطراز التزاوجى *b* بين وخلال تحت العشائر الأربعة المذكورة وحساب معدل الانسياب الجينى (الهجرة) بين تحت العشائر.

ويمكن تلخليص النتائج المتحصل عليها فى :

1- تم تحديد ثمانية أليلات مختلفة لموقع الطراز التزاوجى *b* للجراثيم الأحادية المعزولة من المناطق المختلفة بتكرارات متساوية تقريبا.

2- كانت الأليلات ب b_1 ، b_3 ، b_8 أعلى الأليلات تكرارا بينما كانت الأليلات b_4 ، b_6 الأقل تكرارا .

3- كان الأليل b_4 غائبا فى العينات المأخوذه من سوهاج بينما كان الأليل b_6 غائبا فى عينات أسيوط والمنيا والوجه البحرى.

4- قيم التنوع الجينى (H_s) على مستوى كل العشائر كانت 85.86 بينما كان متوسط التنوع الجينى خلال تحت العشائر 82.79 وكان التنوع الجينى بين تحت العشائر 3.07 مما يوضح أن غالبية التنوع الجينى كان موجوداً على مستوى العشائر الصغيره.

إستخدم تفاعل البوليمريز المتسلسل المعتمد على مكاثرة قطع (DNA) **PCR_RAPD** الموزعه عشوائيا للتفرقه بين العزلات المختلفه للفطر. واختيرت عشرة عينات من مناطق جغرافية مختلفة وتحمل أليلات *b* مختلفة فنتج أربعين حزمه بمعدل 8 حزم لكل بادئ وتراوح حجم هذه الحزم ما بين 150 إلي 2500 زوج من القواعد وكان من بينها عدد 27 حزمه متعددة الأشكال بنسبة 67.50%.

وظهرت حزمه تميز الطراز التزاوجى (a_2) عن الطراز a_1 عن طريق البادئ (OPO-12) وحجمها 2400 زوج من القواعد.

أظهرت النتائج أنه لا توجد علاقة بين أنماط (RAPD) والمواقع الجغرافية المنتمية إليها العزلات المختلفة.