MOLECULAR AND MORPHOLOGICAL VARIATIONS OF SOME CYTOPLASMIC MALE STERILE LINES AND THEIR MAINTAINERS IN RICE

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ABSTRACT

Molecular and morphological variations were studied to detect the genetic diversity between four cytoplasmic male sterile (*CMS*) lines, their four maintainer lines and two local cultivars of rice *(Oryza sativa)* at the mitochondrial DNA (mtDNA) level. Undigested mtDNA from all lines and local cultivars possessed broad band of large molecular weight corresponding to the main mtDNA, and four discrete plasmid like DNA molecules of 2.3, 1.6, 1.5 and 1.2 Kb in size, whereas the local cultivars "*Giza181*" possessed three plasmid like DNA molecules of 2.3, 1.5 and 1.2 Kb, and "*Giza 177*" possessed two plasmid like DNA molecules of 1.5 and 1.2 Kb. Using three 6- base cutter restriction enzymes; *Sal*I, *Kpn*I and *EcoR*I, a total of 43 restriction fragments were observed. Five mtDNA haplotypes were found using *Sal*I, eight haplotypes using *Kpn*I and 27 haplotypes using *EcoR*I. The estimates of nucleotide substitution rates between the different haplotypes ranged from 0.1251±0.0509 to 0.0078±0.0065. The nucleotide substitution rates ranged from 0.0039 ± 0.0033 to 0.0078±0.0065. Also the nucleotide substitution rates between the indica lines and japonica cultivar "*Giza177*" was proven to be 0.0380±0.0340. The nucleotide divergence *(π)* between the *CMS* lines was found to be 0.00527 and between the maintainer lines was shown to be 0.00438. The time since divergence between the indica and japonica subspecies was estimated to be 0.0224 million years ago. Phylogenetic trees have been constructed for the different lines under study using the Neighbor-Joining method.

Seven morphological characters were also measured; plant height, number of tillers per plant, flag leaf length, flag leaf width, panicle length, panicle excision and heading date. All studied morphological characters revealed high significant variations

between all genotypes. Each maintainer line was found to overcome its *CMS* line for the characters of plant height, number of tillers per plant, flag leaf length, flag leaf width, panicle length and panicle excision. The *CMS* lines surmounted the maintainer lines in the heading date character. The local cultivars possessed medium values for all the characters under investigation except for panicle excision character, where local cultivars possessed higher values than the CMS lines, while the local cultivar "Gizal77" surmounted all other genotypes in heading date character.

Key words: *Oryza sativa* - *CMS* - mtDNA - molecular variation – phylogenetics – morphological characters.

INTRODUCTION

When the United Nation's Food and Agriculture Organization chose to dedicate 2004 as the International Year of Rice, it set as a theme, "rice is life," and with good reason. Rice supplies 20% of the world's nutritional energy and is a staple food for more than half the population. Nearly 1 billion households in Asia, Africa and the Americas depend on rice for employment and livelihood; and about four-fifths of the world's rice is produced by small-scale farmers and consumed locally (Maher, 2004). With its genome size of 430 megabase pairs (Mb), the cultivated rice species *Oryza sativa* is a model plant for genome research. The completion of the sequencing of the rice nuclear and cytoplasmic genomes has helped in answering questions related to domestication, speciation, and ecological adaptation (Vaughan *et al.*, 2003). Research groups within the Beijing Genomics Institute's Rice Information System published presented sequenced, annotated genomes for both indica and japonica for in-depth comparative studies. (Zhao *et al.*, 2004 and Yazaki *et al.*, 2004).

Scientists were unable to make hybrid seed because rice is a self- pollinating crop. Wild rice is often referred to as wild rice with abortive pollen, or WA. They soon succeeded in transferring the male sterility trait, known as Cytoplasmic Male Sterility (CMS) (A line) to cultivated varieties. It was found that some varieties, if crossed to a CMS line, produce plants that are also male sterile. These lines, called maintainer lines (B line), were used to keep the male sterile trait. Other lines – referred to as restorer lines (R line) as they restore fertility – if crossed to a CMS-line result in fertile

plants. The first hybrid rice was released in China in 1976, and accounted for approximately 90% of hybrid rice (Yuan, 1992 and Huang *et al.*, 2002).

CMS is maternally inherited and controlled by the mitochondrial genes. It is caused by lesion or rearrangement of mitochondrial genome, but can be restored by nuclear genes (Shinjyo and Sato, 1994 and Zhang, 1997) and was mapped on different chromosomes (Shinjyo and Sato, 1994; Bharaj *et al.*, 1995; Yao *et al.*, 1997; Zhang *et al.*, 1997 and Jing *et al.*, 2001). Research on the molecular biology of rice mitochondrial DNA (mtDNA) has expanded recently (Laughnan and Gabay-Laughnan, 1983; Newton, 1988, Harai *et al.*, 1990 and Sun *et al.*, 2002). In petunia, a mitochondrial gene was cloned which encodes CMS and an abnormal protein which disrupts mitochondrial activities. However, a nuclear gene was found to restore normal fertility to plant genotypes (Bentolila *et al.*, 1998 and Hanson *et al.*, 1999). Moreover, there is evidence for intragenic recombination in the history of the haplotype sample, implying at least transient heteroplasmy of mitochondrial DNA (mtDNA). Heteroplasmy might be achieved by one of two potential mechanisms, either continuous coexistence of subgenomic fragments in low stoichiometry, or occasional paternal leakage of mtDNA. On the basis of levels of synonymous nucleotide substitutions, the average divergence time between haplotypes is estimated to be at least 15 million years. Ancient coalescence of extant haplotypes is further indicated by the paucity of fixed differences in haplotypes (Matsuoka *et al.*, 2002).

In maize (*Zea mays*), there are two genes; rf2a and rf2b. The RF2A protein was shown previously to accumulate in the mitochondria. The rf2a gene was found to participate in normal anther development and the restoration of Texas cytoplasm-based male sterility. This gene; rf2a, can restore fertility by preventing premature programmed cell death (Liu and Schnable, 2002).

Recent reports using Southern hybridization and the RFLP (restriction fragment length polymorphism) techniques on mtDNA in rice suggested that there are common rearrangements in mtDNA which create many RFLP variations (Kadowaki *et al.*, 1986. and 1989, Sakamoto, 1991 and Abe *et al.*, 1999). Further reports related

the presence of plasmids in the construction of mtDNA with the consequence of CMS lines in rice (Yamaguchi *et al.*, 1986). Yashitola *et al.* (2004) reported the identification of a DNA sequence that is homologous to rice mitochondrial DNA but unique to the Wild Abortive (WA) cytoplasmic male sterile lines of rice.

Mitochondrial DNA analysis has been utilized to determine the molecular variation within natural population or species related to the same genera (Avise and Lansman, 1983; Nei, 1987 and Megeed *et al.*, 1998).

It is worth mentioning that this study is the first to be conducted in Egypt at the mtDNA level of rice CMS lines and their maintainers as well as the two Egyptian local cultivars. This study was carried out to accomplish the estimation of genetical and molecular variations of the mtDNA haplotypes for the CMS lines under study, their maintainer lines, and the Egyptian local cultivars.

MATERIALS AND METHODS

The present investigation was carried out at the Department of Genetics, and the Experimental Farm, Faculty of Agriculture, Kafr- EL-Sheikh, Tanta Univ. Egypt.

I-Plant Samples:

Ten different lines of rice (four CMS lines, four maintainer lines and two Egyptian cultivars) were used in this investigation. The four CMS lines are IR67701A, IR68276A, IR68277A and IR68884A. The four maintainer lines are IR67701B, IR68276B, IR68277B and IR68884B. The Egyptian cultivars are Giza181 as Indica type with long grain and Giza177, which belongs to the Japonica type, with short grain. These lines were provided by the Rice Research and Training Center (R.R.T.C.), Sakha, Kafr EL-Sheikh, Egypt. The characterization of the used genotypes is illustrated in Table (1).

II-Mitochondrial DNA (mtDNA) techniques:

II-1-Extraction and purification of mitochondria for DNA analysis:

Seeds were soaked 45 min in demestose belch, washed six times with sterile water and germinated in humid sterile soil consisted of potmos and sand (3:1 v/v). The seeds were allowed to be grown in the dark for ten days at 30°C.

Table (1). Origin and salient features of the four CMS lines, their maintainers, and the two Egyptian cultivars used in this study.

Genotypes	Origin (parentage)	Salient features
IR67701A	IRRI Acces.. No CMS 14977	Indica type, early maturing, medium tall, short grain.
IR68276A	IRRI Acces.. No CMS 15027	Indica type, very early maturing, medium tall, short grain.
IR68277A	IRRI Acces.. No CMS 14987	Indica type, very early maturing, medium tall, short grain.
IR68884A	IRRI Acces.. No CMS 15019	Indica type, medium maturing, medium tall, medium grain.
IR67701B	IRRI Acces.. No CMS 14978	Indica type, medium maturing, medium tall, short grain.
IR68276B	IRRI Acces. No CMS 15028	Indica type, early maturing, medium tall, short grain.
IR68277B	IRRI Acces. No CMS 14988	Indica type, early maturing, medium tall, short grain.
IR68884B	IRRI Acces. No CMS 15020	Indica type, medium maturing, medium tall, medium grain.
Giza177	Egypt (Giza 171/ Yamji No. 1 //pi. No 4	Japonica type, very early maturing, semi-dwarf, short grain, resistant to blast and high yielder.
Giza181	IRRI-Egypt (IR24 ×IR 22)	Indica type, medium maturing, semi-dwarf, resistant to blast and excellent long grain and high yielder.

Mitochondria were isolated as described by Saleh *et al.* (1989) with some modifications, which are reported here for the first time. Twenty grams of leaves were washed and cut into small pieces. Then the tissues were homogenized with chilled mortar and pestle in 100 ml buffer A (0.5 M mannitol, 10 mM TES (pH 7.4), 0.2% BSA and 0.05% cystein). The homogenate was filtered through four layers of cheesecloth and centrifuged at 1500 rpm for 10 min (IEC189 rotor). The supernatant was transferred to a new tube and centrifuged at 12000 rpm for 20 min. Then the pellet was re-suspended gently in buffer A and layered into gradient sucrose (1.8, 1.5, 1.2 and 0.6 M) and centrifuged at 15000 rpm for 45 min. The mitochondria could be located between 1.5 M and 1.2 M. This phase was taken with micropipette or 10 ml syringe and put in a clean tube. Equal volume of buffer A was then slowly added to the

tube and centrifuged at 12000 rpm for 10 min. The pellet was re-suspended in 1 ml of buffer B (0.01 M Tris-Hcl (pH 8.0), 0.1 M NaCl, 0.05 M EDTA, 0.2 M sucrose and 0.5 M SDS) and gently mixed. After incubation for 30 min at 65 °C. 1000µL of 8M Potassium acetate were added and mixed. The re-suspension was incubated on ice for 30 min. At this step potassium acetate and SDS formed a whitish mass that floated. The re-suspension was subjected to flicks from time to time for good mixing. Then 100µL of sterile water was added. The mixture was centrifuged 15 min in the microcentrifuge at 14000 rpm. Supernatant was transferred to a clean tube. Then, 332µL of 7.5 M ammonium acetate were added and spanned at 14000 rpm for 15 min. To ensure that purification of mtDNA from protein, the supernatant was treated three cycles with phenol:chloroform:isoamyle alcohol (25:24:1) mixture. Aqueous phase was then transferred to another tube and mtDNA was precipitated by adding 2.5 volumes of 80% cold ethanol (3 ml) and then the mixture was left overnight, then spun for 30 min in the microcentrifuge, washed with 70% ethanol, then vortex to remove the salt and, then, centrifuged for 5 min. This step was repeated two to four times if there is a great deal of salt. Supernatant was removed carefully and air dry. Pellet was re-suspended in 20 –50µL of TE buffer (10 mM Tris-HCl, pH 8.0, and 1mM EDTA pH 8.0).

II-2- Restriction enzymes digest and agarose gel electrophoresis:

Three restriction endonucleases (6-base cutters), *Kpn* I, *Sal* I and *EcoR* I were used. These enzymes and their buffers were obtained from Boehriger Mannheim Ltd. Co., Germany. The enzymes, their recognition sites and the used buffers are shown in Table (2).

Table (2). Restriction enzymes, their recognition sites and the used buffers.

Enzyme	Recognition site	Buffer	Buffer contents			
			NaCl	MgCl	Tris-HCl	DTT
EcoR I	5`–G↓AATTC-3`	HSB	100mM	10mM	50mM	1mM
Sal I	5`-C↓TCGAG-3`	HSB	100mM	10mM	50mM	1mM
Kpn I	5`-GGTAC↓C-3`	LSB	10mM	10mM	50mM	1mM

The arrows indicate the position at which cleavage occurs. HSB=High Salts Buffer. LSB=Low Salts Buffer.

Mixing the following components in a sterile eppendorf tube assembled the reactions, according to Sambrook *et al.* (1989): mtDNA; about 1 µg, 10X buffer; 5 µL, and enzyme 3-5 units.

Sterile bidistilled water was added to a final volume of 20µL. The reactions were incubated at 37°C for 3 h, 5 µL of stop solution (0.05% bromophenol blue, 50 mM EDTA and 0.05% SDS) were added to stop the reaction.

The DNA samples were loaded on 1% agarose gels with TBE running buffer (10.8 g Tris, 5.5 g boric acid and 0.75 g EDTA up to 1000 ml D.W., pH 8.0). After electrophoresis, the gels were stained with ethidium bromide (0.5µg/ml) for 30 min and destained with water for 30 min, and the mtDNA bands were visualized under UV transeleminator plate. Gels were photographed using Polaroid camera DS34. Sizes of restriction fragments were estimated using a graphical method using *Hind III* digested lambda phage DNA as a size marker.

II-3. Statistical procedures for molecular mtDNA variations:

Estimates of nucleotide diversity (nucleotide substitution rate; d_{ij}, the average of the number of nucleotide substitutions per site between two sequences), the net nucleotide divergence rate (d_A) and nucleotide diversity (π) were calculated using the computer program "RESTSITE" (Nei and Miller, 1990) and according to Nei (1987) and as follows: $d_{ij} = -3/4 \log_e (1 - (4p_{ij} / 3))$, *where* p_{ij} *is the average of the proportion of different nucleotides between two sequences over all pairwise comparisons.* $d_A = d_{xy} - (d_x + d_y) / 2$, *where* d_x *is the nucleotide substitution rate of the population (x) and* d_y *is the nucleotide substitution rate of the population (y) and*

$$\pi = 2 \sum_{i<j} d_{ij} / n (n - 1)$$

The time since divergence was estimated according to Nei (1987), Saitou and Nei (1987) and Nei and Jin (1989) as follows: Since $d = 2\lambda t$, then $t = d / 2\lambda$.

Phylogenetic trees were established using the computer program "RESTSITE" (Nei and Miller (1990) and the method of neighbor-joining (NJ) (Saitou and Nei, 1987).

III-Morphological characters:

The measurements for each character were conducted using

two replicates each with ten plants for each entry with total of 200 plants. The morphological characters were: heading date (in days), plant height (in centimeters), flag leaf length (in centimeters), flag leaf width (in centimeters), panicle length (in centimeters), panicle Excision (as percentage), and number of the fertile tillers per plant.

III-1-Statistical procedures for morphological characters:

The statistical analyses of the four CMS lines data, their maintainer lines as well as the two Egyptian cultivars were carried out using the analysis of variance and L.S.D to test the significance of the differences between means according to Sendecor (1958).

RESULTS AND DISCUSSION

1. Mitochondrial DNA (mtDNA) studies:

Recent advances in molecular breeding methods hold tremendous potential for genetic improvement of rice cultivars with beneficial genes from wild rice species. A clear understanding of the evolutionary relationships of rice species will be essential in directing these efforts to search for such genes.

Since this study is considered to be the first on rice mtDNA in Egypt, one of the objectives of this study is to enlighten the breeding programs for the powerful advantages of RFLP technique to estimate the molecular variation in natural populations as well as species and to elucidate the concepts of molecular evolution.

In rice, mitochondria of S_1 CMS lines have been shown to possess two (Yamaguchi and Kakuchi, 1983) or four (Kadowaki *et al.*, 1986) plasmid like molecules in addition to the main mtDNA. In mitochondria associated with S_2 CMS, four plasmid like DNAs with low molecular weight, were identified (Mignouna *et al.*, 1987). Several research works argued about whether this plasmid like DNA affect or do not affect the incidence of CMS.

1.1. Sizes and patterns of restriction fragments:

Unlike the plastid genomes of angiosperms, which are relatively constant in size and coding function, mitochondrial genomes of higher plants were found to be very divergent in size and sequence content (Newton, 1988). The smallest known mitochondrial DNA (mtDNA) in an angiosperm was the 208 Kb genome found in *Brassica hirta* (Palmer and Herbon, 1987). While the rice mitochondrial genome was 500 Kb (Harai *et al.*, 1990),

300Kb (Wang *et al.*, 1989) and from 350 to 450 Kb (Shikanai *et al.*, 1987), it was very unlike that rice mtDNA encoded more genes than the *Brassica* genome. In fact, despite their larger sizes, the mtDNAs in higher plants contain fewer genes than the chloroplast DNA (ctDNA).

Rice mitochondrial DNA consists of four kinds of circular DNA. Each circular DNA shares homologous sequences with one to three of other circular DNAs. If recombinations occur in homologous sequences, the known kinds of circular DNA would be observed. This explains the multicircular organization of mtDNA in rice, which the various circular molecules are presented in different stoichiometric amounts (Kanazawa *et al.*, 1998).

Data in Figure (1a) show that all the CMS lines and their maintainer lines possess four discrete plasmid like DNA molecules of 2.3, 1.6, 1.5 and 1.2 Kb in size as measured relatively to the linear marker DNA bands. This result is in agreement with Saleh *et al.* (1989) and Mignouna *et al.* (1987). On the other hand, the local cultivar Giza181 possessed three plasmid like DNA molecules of 2.3, 1.5 and 1.2 Kb but Giza177 the local cultivar was found to possess two plasmid like DNA molecules of 1.5 and 1.2 Kb.

The basic restriction fragment maps reveal the different fragment patterns and sizes of the fragments, which have been detected for the *Kpn I*, *Sal* I and *EcoR I* digested mtDNAs in the four CMS lines, their four maintainer lines and the two local cultivars (Figures 1b-1g and Tables 3-4). A total of 43 restriction fragments were scored in the four CMS lines, their four maintainer lines and the two local cultivars using the three restriction enzymes; *Kpn I*, *Sal I* and *EcoR I*. Thirty nine fragments out of the 43 were polymorphic (90.7%). Lu *et al.* (2002) scored an average of 3.8 polymorphic fragments per probe in 58 accessions of *O. rufipogon*, *O. nivara*, *O. sativa f. spontanea* and the cultivated *O. sativa*. Park *et al.*, (2003) detected a total of 250 polymorphic fragments from the analysis of 53 accessions of *Oryza* species with seven MITE-AFLP primer combinations.

Nine fragments were scored for the *Kpn* I digest (Table 3). While seven fragments were scored for each of the lines IR67701A, IR68276A, IR68277A, IR68884A, IR67701B, and IR68276B, there were four fragments scored for the line

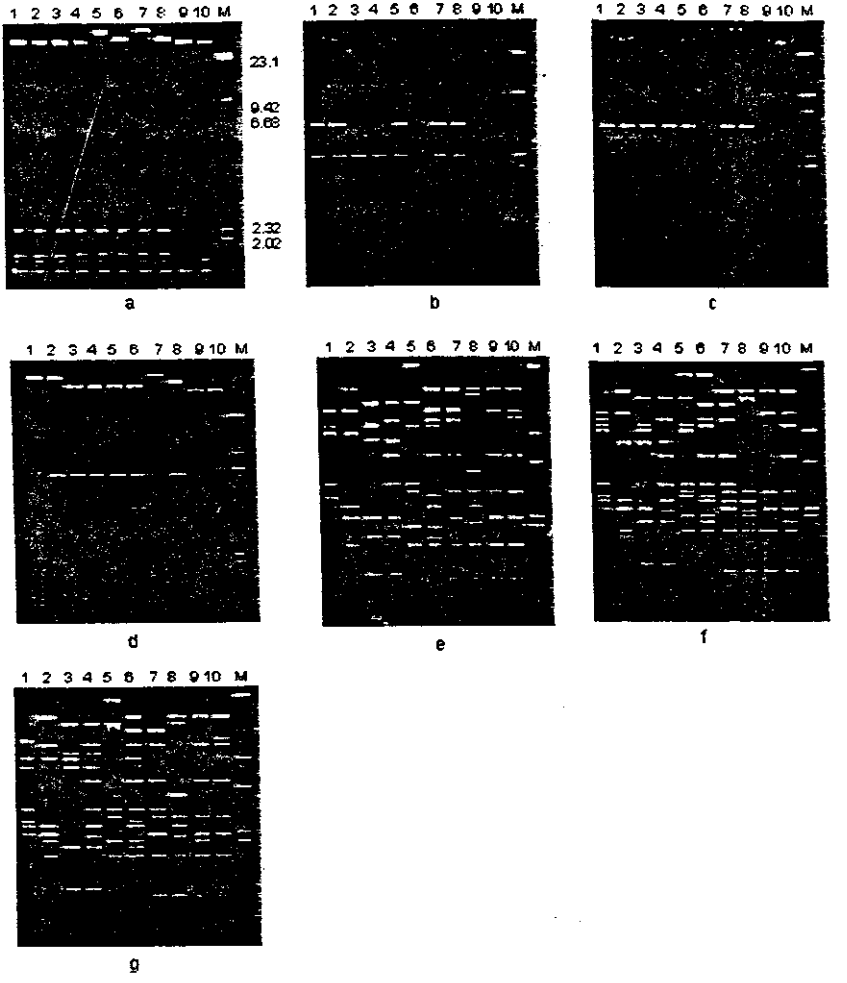

Figure (1). Polaroid photographs of agarose gel electrophoresis of rice mtDNA, a- Undigested mtDNA, b-c- mtDNA digested with Kpn1, d- mtDNA digested with Sal1,and e-g- mtDNA digested with EcoR1,Lane1-IR67701A, Lane2- IR67701B, Lane3- IR68276A, Lane4-IR68276B, Lane5 IR68277A, Lane6-IR68277B, Lane7-IR68884A, Lane8-IR68884B, Lane9-Giza177, Lane10- Giza181 and lane 11- DNA marker II.

IR68277B, five fragments for the line IR68884B, and just one fragment for each of the two local cultivars Giza177 and Giza181. The data in Table (3) show that the *Kpn* I digest gave eight different fragment patterns (A, B, C, D, E, F, G, H and I). These eight patterns are corresponding to eight different haplotypes. These eight haplotypes were distributed among the thirty mtDNAs representing the rice lines under investigation.

The two maintainer lines; IR68277B and IR68884B, were shown to be monomorphic and they possessed two different haplotypes. Also, the two local cultivars; Giza177 and Giza188 were found to be monomorphic, but they possessed one haplotype.

The number of polymorphic fragments was 7 (77.78%).The nine fragments scored in this study for the *Kpn* I digest have never been reported by any other author (Ishii *et al* 1992 and Shikanai and Yamada, 1988) and they are reported here for the first time.

In the case of *Sal* I digest there were five different fragments with the sizes of 55, 49, 47, 7.95 and 3.95 Kb. This digest with *Sal*1 gave five different patterns (Table 3). All the five fragments scored in this digest were polymorphic.

In the case of the digest with the restriction enzyme *EcoR I,* this digest gave 29 polymorphic fragments and 27 haplotypes (Table 4). Except for the maintainer lines; IR68276B and IR68884, and the local cultivar Giza181, all the other lines gave three different patterns.

1.2. Restriction Fragments Variations:

The number of nucleotide substitutions within each line ranged from 0.0030±0.0026 (the maintainer line IR68884B) to 0.0126±0.0041 (the CMS line IR68276A) (Table 5). This means that this CMS line is highly polymorphic and showed a weakened constraint against the changes in its mtDNA sequences along the time of its evolution. On the other hand, the maintainer line; IR68884B, showed that it was highly constrained against the nucleotide substitutions in its mtDNA that may occurr along its evolutionary pathway, or it is recently diverged from another older line. The two CMS lines IR67701A and IR68276A had the highest nucleotide substitution rates between each of them and all the other lines, except for the two cases with their maintainer lines. The

Table (3). Restriction fragment map of mtDNA for each of the 30 haplotypes of the four CMS lines, their maintainer lines and the two local cultivars digested with *Kpn*1 and *Sal*1.

Haplotypes	Pattern	*Kpn*1 fragments									Pattern	*Sal*1 fragments				
		28	25.6	23.8	4.8	2.3	0.45	0.41	0.35	0.3		55	49	47	7.95	3.95
IR67701A-1	A	-	+	-	+	+	-	-	+	+	A	-	+	-	+	-
-------- -2	B	-	+	-	+	+	+	+	+	+	A	-	+	-	+	-
-------- -3	A	-	+	-	+	+	-	-	+	+	A	-	+	-	+	-
IR68276A-1	C	-	-	+	-	+	-	-	-	+	B	-	-	+	+	+
-------- -2	D	-	-	+	+	+	+	+	+	+	B	-	-	+	+	+
-------- -3	C	-	-	+	-	+	-	-	-	+	B	-	-	+	+	+
IR68277A-1	A	-	+	-	+	+	-	-	+	+	B	-	-	+	+	+
-------- -2	B	-	+	-	+	+	+	+	+	+	B	-	-	+	+	+
-------- -3	A	-	+	-	+	+	-	-	+	+	B	-	-	+	+	+
IR68884A-1	E	+	-	-	+	+	-	-	+	+	C	+	-	-	-	-
-------- -2	F	+	-	-	+	+	+	+	+	+	C	+	-	-	-	-
-------- -3	E	+	-	-	+	+	-	-	+	+	C	+	-	-	-	-
IR67701B-1	A	-	+	-	+	+	-	-	+	+	A	-	+	-	+	-
-------- -2	B	-	+	-	+	+	+	+	+	+	A	-	+	-	+	-
-------- -3	A	-	+	-	+	+	-	-	+	+	A	-	+	-	+	-
IR68276B-1	C	-	-	+	-	+	-	-	-	+	B	-	-	+	+	+
-------- -2	D	-	-	+	+	+	+	+	+	+	B	-	-	+	+	+
-------- -3	C	-	-	+	-	+	-	-	-	+	B	-	-	+	+	+
IR68277B-1	G	+	-	-	-	+	-	-	+	+	B	-	-	+	+	+
-------- -2	G	+	-	-	-	+	-	-	+	+	B	-	-	+	+	+
-------- -3	G	+	-	-	-	+	-	-	+	+	B	-	-	+	+	+
IR68884B-1	E	+	-	-	+	+	-	-	+	+	D	-	+	-	+	+
-------- -2	E	+	-	-	+	+	-	-	+	+	D	-	+	-	+	+
-------- -3	E	+	-	-	+	+	-	-	+	+	D	-	+	-	+	+
Giza177-1	H	-	+	-	-	-	-	-	-	-	E	-	-	+	-	-
-------- -2	H	-	+	-	-	-	-	-	-	-	E	-	-	+	-	-
-------- -3	H	-	+	-	-	-	-	-	-	-	E	-	-	+	-	-
Giza181-1	H	-	+	-	-	-	-	-	-	-	E	-	-	+	-	-
-------- -2	H	-	+	-	-	-	-	-	-	-	E	-	-	+	-	-
-------- -3	H	-	+	-	-	-	-	-	-	-	E	-	-	+	-	-

(+) = presence of mtDNA fragments and (-) = absence of mtDNA fragments.

Table (4)- Continued.

Haplotypes	Pattern	Fragments												
		2.2	2	1.7	1.3	0.92	0.84	0.78	0.7	0.64	0.54	0.44	0.3	0.27
IR67701A-1	A-1	-	-	-	+	-	-	-	-	-	+	-	-	-
——— -2	B-2	-	+	-	+	-	-	-	-	-	+	-	-	-
——— -3	C-3	-	+	-	+	-	-	-	-	-	+	-	-	-
IR68276A-1	D-4	-	-	+	-	+	-	-	-	-	-	-	-	-
——— -2	E-5	-	-	+	-	+	-	-	-	+	-	-	-	-
——— -3	F-6	-	-	+	-	+	-	-	+	+	-	+	-	-
IR68277A-1	G-7	-	+	-	+	-	-	+	-	-	+	-	-	+
——— -2	H-8	-	+	-	+	-	-	+	-	-	+	+	-	+
——— -3	I-9	-	+	-	+	-	-	+	-	+	+	-	-	+
IR68884A-1	J-10	-	-	-	+	-	+	-	+	-	-	-	-	-
——— -2	K-11	-	-	-	+	-	+	-	+	-	-	-	-	-
——— -3	L-12	-	-	-	+	-	+	-	+	-	-	-	-	-
IR67701B-1	M-13	-	-	-	+	-	+	-	-	+	+	-	-	+
——— -2	N-14	-	-	-	+	-	+	-	-	+	+	-	-	+
——— -3	O-15	-	+	-	+	-	+	-	-	+	+	-	-	+
IR68276B-1	P-16	+	-	+	-	+	-	-	-	-	-	-	-	-
——— -2	P-16	+	-	+	-	+	-	-	-	-	-	-	-	-
——— -3	Q-17	+	-	+	-	+	-	-	-	-	+	+	-	-
IR68277B-1	R-18	-	+	+	+	-	-	+	-	-	-	-	-	-
——— -2	S-19	-	+	+	-	+	-	+	-	-	-	+	-	-
——— -3	T-20	-	+	+	+	-	-	+	-	-	-	+	-	-
IR68884B-1	U-21	+	-	-	+	-	+	-	-	-	+	+	+	-
——— -2	V-22	+	+	-	+	-	+	-	-	-	+	+	+	-
——— -3	U-21	+	-	-	+	-	+	-	-	-	+	+	+	-
Giza177-1	W-23	-	-	-	+	-	+	-	-	-	-	-	-	-
——— -2	X-24	-	-	-	+	-	+	-	-	-	-	-	+	-
——— -3	Y-25	-	+	-	+	-	+	-	-	-	-	-	-	-
Giza181-1	Z-26	-	-	-	+	-	+	-	-	-	-	-	-	-
——— -2	AA-27	-	-	+	+	-	+	-	-	-	-	-	-	-
——— -3	Z-26	-	-	-	+	-	+	-	-	-	-	-	-	-

(+) presence of mtDNA fragments (-) absence of mtDNA fragment.s

Table (4). Restriction fragment map of mtDNA for each of the 30 haplotypes digested with, the restriction enzyme; *EcoR*1.

Haplotypes	Pattern	Fragments															
		20	17	15.5	14	13	11	10	9.4	8.5	7.2	5.6	4.7	3.9	3.6	3	2.3
IR67701A-1	A-1	-	-	-	-	+	-	+	+	-	-	-	+	-	+	+	+
——— -2	B-2	-	-	-	-	+	+	+	+	-	-	-	+	-	+	+	+
——— -3	C-3	-	-	-	-	+	-	-	+	+	-	-	+	-	+	+	+
IR68276A-1	D-4	-	-	+	-	-	-	+	-	+	-	-	-	-	-	-	+
——— -2	E-5	-	-	+	-	-	-	+	+	+	-	-	-	-	-	-	+
——— -3	F-6	-	-	+	-	-	-	+	+	+	-	-	-	-	-	-	-
IR68277A-1	G-7	+	-	+	-	-	-	+	-	-	-	-	+	+	-	-	-
——— -2	H-8	+	-	+	-	-	-	+	+	-	-	-	+	+	+	-	-
——— -3	I-9	+	-	+	-	-	-	-	-	-	-	-	+	+	-	-	-
IR68884A-1	J-10	-	+	-	+	-	+	-	-	-	+	-	+	+	-	-	+
——— -2	K-11	-	+	-	+	-	+	-	-	-	+	-	+	+	-	+	+
——— -3	L-12	-	-	-	+	-	+	-	-	-	+	-	+	+	-	-	+
IR67701B-1	M-13	-	+	-	-	+	-	-	+	-	-	-	-	-	-	+	+
——— -2	N-14	-	+	-	-	+	-	-	-	+	-	-	-	-	-	+	+
——— -3	O-15	-	+	-	-	-	+	-	+	-	-	-	-	-	-	+	+
IR68276B-1	P-16	-	-	+	-	-	+	-	-	+	+	-	+	-	-	+	-
——— -2	P-16	-	-	+	-	-	+	-	-	+	+	-	+	-	-	+	-
——— -3	Q-17	-	-	+	-	-	+	+	-	+	+	-	+	-	-	+	-
IR68277B-1	R-18	-	+	-	+	-	+	+	-	-	+	-	+	-	+	+	-
——— -2	S-19	-	+	-	+	-	+	+	-	-	+	-	+	-	+	+	-
——— -3	T-20	-	+	-	+	-	+	-	+	-	+	-	+	-	+	+	-
IR68884B-1	U-21	-	+	+	-	-	-	-	-	-	-	+	-	+	-	+	-
——— -2	V-22	-	+	+	-	-	-	-	-	-	-	-	-	+	+	+	-
——— -3	U-21	-	+	+	-	-	-	-	-	-	-	+	-	+	-	+	-
Giza177-1	W-23	-	+	-	-	+	-	-	-	-	+	-	-	+	-	-	+
——— -2	X-24	-	+	-	-	+	-	-	-	-	-	-	-	+	-	-	+
——— -3	Y-25	-	+	-	-	-	+	-	-	-	+	-	-	+	-	-	+
Giza181-1	Z-26	-	+	-	-	+	+	-	-	-	+	-	-	+	-	-	+
——— -2	AA-27	-	+	-	-	+	-	+	-	-	+	-	-	+	-	-	+
——— -3	Z-26	-	+	-	-	+	+	-	-	-	+	-	-	+	-	-	+

study shows that the two maintainer lines, IR67701B and IR68276B, are the most distinct lines between the maintainer lines (0.0660 ±0.290). This will be also revealed by the phylogenetic analysis that these two sequences are less conserved in evolution compared to the highly conserved chloroplast genome.

Caccone *et al.* (1988) calibrated their divergence rate using biogeographical and geological information on divergence times. They proposed a substitution rate of 1.7% base substitution per million years since last common ancestor, or 0.85% per million years per lineage. Using this value, the time since divergence between the CMS lines and their maintainers could be estimated using the equation $d = 2\lambda t$ (Nei, 1987).

The estimated nucleotide substitution rates between the CMS lines and their maintainers and between *Oryza indica* and *Oryza japonica* in this study were 0.0039 and 0.0380, respectively (Table 6). So, the time since divergence (t) between the CMS lines and their maintainers is 0.0023 million years ago and 0.0224 million years ago between *Oryza indica* and *Oryza japonica.*

1.3. Phylogenetic Tree:

Geneticists anticipated that cultivated rice, *Oryza sativa*, will soon become the first crop plant with its entire genome sequenced. Clarifying evolutionary relationships among genome types of rice species will provide a foundation for future studies of rice genome evolution.

Phylogenetic studies of *Oryza*, however, have been less extensive than those of other major crop plants, such as maize, soybean, and cotton. Evolutionary relationships among the rice genomes and species were previously estimated by phenetic analysis of morphology, isozyme, nuclear and chloroplast DNA restriction fragment-length polymorphisms (Ge *et al.*, 1999, Bautista *et al.*, 2002 and Cheng *et al.*, 2002). However, limitations in the nature of the data and/or methods of data analysis in these studies have hampered an accurate reconstruction of the rice phylogeny. This study used, for the first time in Egypt, the mtDNA to investigate the molecular evolutionary relationships between the CMS lines and their maintainers and to study the rice phylogeny.

Table (5). Estimates of net nucleotide substitutions per fragment of mtDNA between the haplotypes of CMS lines, their maintainer lines and the local cultivars (d_A) (below the diagonal), nucleotide substitution rate within each line (di) (on the diagonal), and the nucleotide substitution rates between each two lines (dij) (above the diagonal).

Haplotypes	IR67701 A (1)	IR68276 A (2)	IR68277 A (3)	IR68884 A (4)	IR67701 B (5)	IR68276 B (6)	IR68277 B (7)	IR68884 B (8)	Giza 177 (9)	Giza 181 (10)
IR67701A (1)	0.0067± 0.0015	0.0575± 0.0207	0.0306± 0.0189	0.0458± 0.0230	0.0180± 0.0137	0.0534± 0.0156	0.0472± 0.0295	0.0386± 0.0221	0.0736± 0.0203	0.0691± 0.0237
IR68276A (2)	0.0478± 0.0207	0.0126± 0.0041	0.0446± 0.0267	0.0858± 0.0614	0.0604± 0.0238	0.0226± 0.0142	0.0472± 0.0295	0.0629± 0.0334	0.1251± 0.0509	0.1050± 0.0265
IR68277A (3)	0.0237± 0.0180	0.0348± 0.0246	0.0071± 0.0024	0.0548± 0.0318	0.0362± 0.0253	0.0454± 0.0267	0.0350± 0.0182	0.0345± 0.0148	0.0730± 0.0133	0.0770± 0.0165
IR68884A (4)	0.0401± 0.0234	0.0772± 0.0631	0.0489± 0.0320	0.0047± 0.0014	0.0414± 0.0175	0.0600± 0.0170	0.0368± 0.0135	0.0406± 0.0287	0.0490± 0.0133	0.0445± 0.0126
IR67701B (5)	0.0107± 0.0055	0.0502± 0.0234	0.0288± 0.0239	0.0351± 0.0177	0.0078± 0.0024	0.0660± 0.0290	0.0542± 0.0189	0.0300± 0.0136	0.0522± 0.0375	0.0452± 0.0411
IR68276B (6)	0.0460± 0.0171	0.0123± 0.0060	0.0378± 0.0266	0.0536± 0.0197	0.0580± 0.0300	0.0081± 0.0042	0.0295± 0.0124	0.0464± 0.0160	0.1189± 0.0380	0.0916± 0.0173
IR68277B (7)	0.0339± 0.0045	0.0384± 0.0265	0.0290± 0.0150	0.0320± 0.0132	0.0478± 0.0160	0.0229± 0.0111	0.0050± 0.0043	0.0390± 0.0251	0.0782± 0.0170	0.0674± 0.0229
IR68884B (8)	0.0337± 0.0202	0.0552± 0.0317	0.0294± 0.0126	0.0367± 0.0284	0.0246± 0.0111	0.0409± 0.0162	0.0351± 0.0218	0.0030± 0.0026	0.0706± 0.0126	0.0797± 0.0123
Giza177 (9)	0.0646± 0.0255	0.1133± 0.0466	0.0639± 0.0079	0.0412± 0.0136	0.0427± 0.0433	0.1093± 0.0350	0.0702± 0.0223	0.0636± 0.0137	0.0111± 0.0092	0.0078± 0.0065
Giza181 (10)	0.0629± 0.0268	0.0959± 0.0237	0.0705± 0.0131	0.0393± 0.0125	0.0452± 0.0411	0.0847± 0.0159	0.0621± 0.0271	0.0753± 0.0130	0.00000	0.0057± 0.0047

Table (6). Estimates of nucleotide substitutions per fragment (d_A) of mtDNA between the haplotypes of CMS, its maintainer, and the local cultivars.

Haplotypes	Maintainer	Giza177	Giza181	*O. indica*
CMS	0.0039±0.0033	0.0486±0.0319	0.0472±0.0329	
Maintainer		0.0529±0.0448	0.0511±0.0461	
Giza177			0.0078±0.0064	
O.japonica				0.0380± 0.0341

The average genetic distance, of mtDNA, among the CMS lines was 0.00527, this estimate is very near to that obtained by Ishii *et al.* (1992), where they estimated it equal to 0.0070. They also found the genetic distance was 0.0065 for the nuclear genome. These high, and equal, estimates of evolutionary variability for both mtDNA and nuclear genomes indicate that enough diversity exists to broaden the genetic base of new cultivars.

The (NJ) method (Figure 2) clustered all the three haplotypes representing the maintainer line; IR67701B, then it joined them with the two branches; one of the three haplotypes representing its corresponding CMS line; IR67701A, and the other branch comes from all the other haplotypes. This means that this maintainer line; IR67701B, is the oldest *O. indica* line. The study found that the closest lines are the CMS line IR68276A and its maintainer line; IR68276B, which were joined together in one cluster. The (NJ) phylogenetic tree joined the last cluster with the maintainer line; IR68277B, but, then, the tree joined this cluster with the CMS line IR68277A. The last cluster was then joined with the maintainer line; IR68884B. The CMS line; IR68884A, which is presumed a relative to this last maintainer line, was clustered first with the two local cultivar; Giza177 and Giza181, and then rejoined with the cluster comes from its maintainer line. These two local cultivars were clustered together before they were joined with the CMS line. This also was confirmed from the (NJ) tree (Figure 3) of the ten lines.

Whereas Abe *et al.* (1999) found that the mtDNA RFLP patterns of *japonica* and *indica* subspecies were clearly different from each other, this study found no molecular variation between the two local cultivars representing the two subspecies. This may be due to the long period adaptation of these local cultivars to the Egyptian environments.

Using the variations detected from the molecular data and the phylogenetic analysis, the study found that the CMS lines could be compared with, and also could be distinguished from, their maintainers.

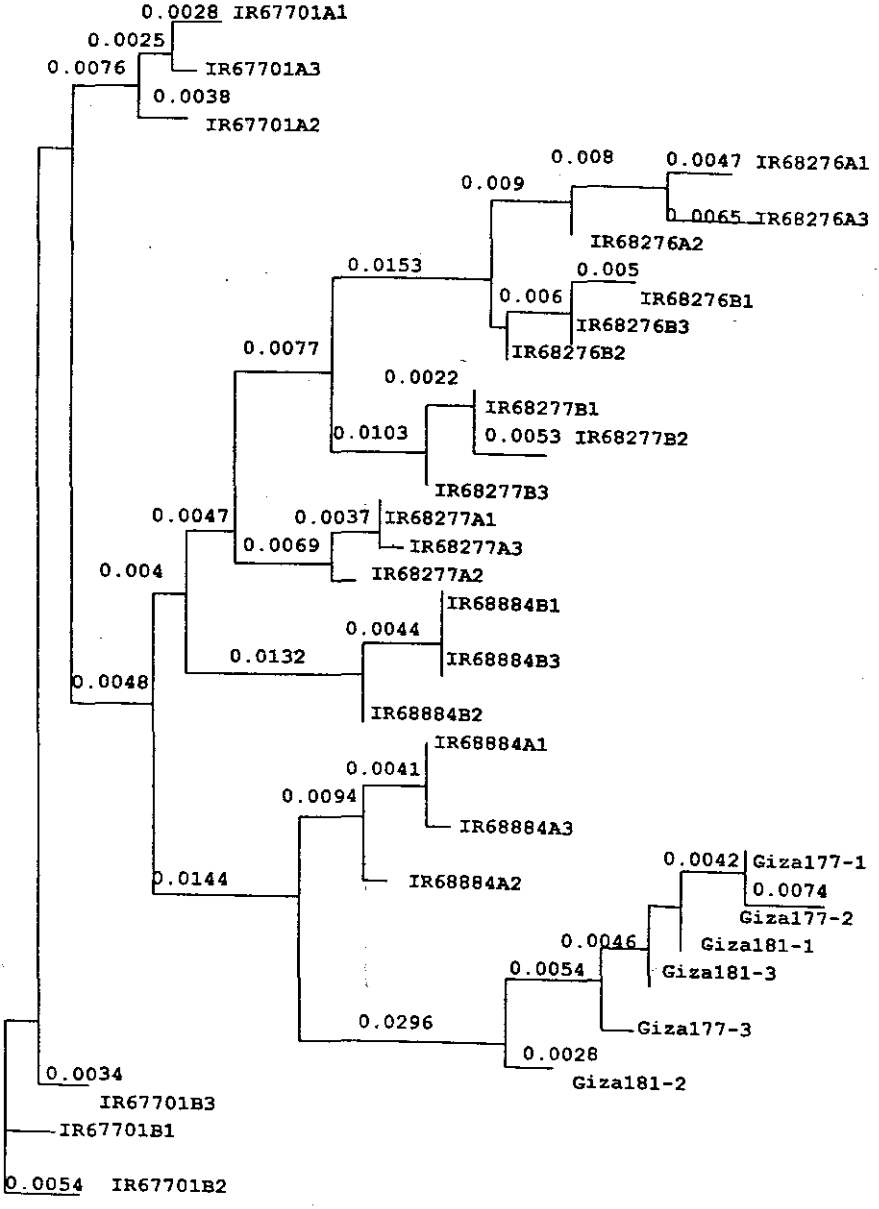

Figure 2. Phylogenetic tree of mtDNA in 30 rice haplotypes; four CMS lines, their four maintainer lines, and two Egyptian cultivars, using the Neighbor-Joining method.

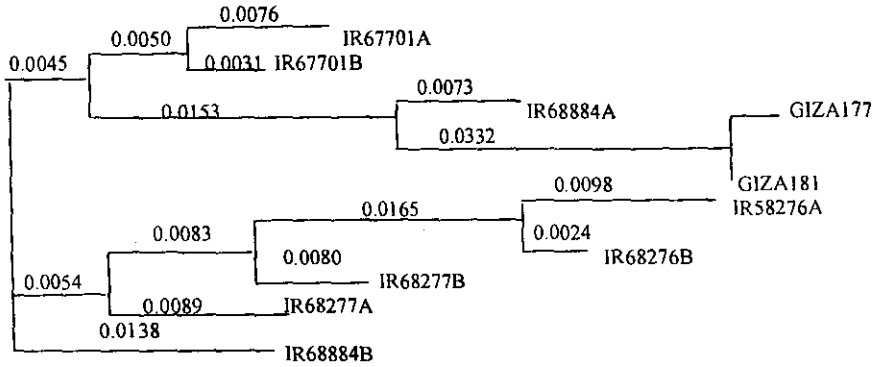

Figure 3. Phylogenetic tree of mtDNA in ten rice lines; four CMS lines, their four maintainer lines and two Egyptian cultivars, using the Neighbor-Joining method.

Yu *et al.* (2003) indicated that indica and japonica differentiation accounted for only 6.5% and 93.5% was due to within-subspecies diversity. Whereas Lu *et al.* (2002) found that the indica rice varieties showed relatively high genetic diversity and the japonica varieties showed a relatively low variation and formed an independent group. They concluded that indica rice is directly domesticated from its ancestral wild species, and japonica rice is derived from indica.

The total gene diversity in six Chinese northern populations of *Oryza rufipogon*, was 0.919 (Song *et al.*, 2003). Analysis of 53 accessions of *Oryza* species detected a total of 250 polymorphic fragments. The genetic distances (GDs) between species were higher than those within species and the GDs in *O. sativa* complex were higher than those in *O. officinalis* complex (Park *et al.*, 2003).

2. Morphological and agronomic characters:

2.1. Plant height:

Results in Table (7) show that the maintainer lines possessed higher estimates than CMS lines, which in turn had higher estimates than the two local cultivars. The plant height measurements for the maintainer lines ranged from 82.10 to 99.15, whereas the corresponding values for the four CMS lines ranged from 78.15 to 96.45. On the other hand, the two Egyptian cultivars; possessed the values of 86.80 and 80.85 cm respectively. Furthermore, differences between mean values of all genotypes were highly significant as shown in Table (8).

2.2. Number of fertile tillers / plant:

Both of IR68276B maintainer line and Giza177 Egyptian cultivar possessed the highest mean estimate of 16.10 for number of fertile tillers /plant, followed by the Egyptian cultivar Giza181, the CMS lines IR68277A and IR68276A and the maintainer lines IR68884B, IR68277B and IR67701B with estimates of 15.85, 15.70, 15.60, 15.10, 14.20 and 12.60, respectively (Table 7). However, the lowest means were 11.90, and 11.55 and were recorded for the CMS lines IR68884A and IR67701A, respectively. Table (8) also indicates that highly significant differences existed either among the genotypes or among the four CMS lines, their maintainer lines, and the two Egyptian cultivars.

2.3. Flag leaf length:

Mean estimates of flag leaf length for the four CMS lines IR67701A, IR68276A, IR68277A, and IR68884A were 25.71, 20.88, 27 and 24.63 cm, respectively (Table 7), however the four maintainer lines IR67701B, IR68276B, IR68277B, and IR68884B showed to possess mean estimates of 28.99, 21.52, 26.03, and 26.80 cm, respectively. On the other hand, the two Egyptian cultivars Giza177, and Giza181 possessed estimates of 22.67, and 28.50 cm, respectively. All the maintainer lines had higher estimates than their corresponding CMS lines; except for the CMS line IR68277A which exhibited mean estimate exceeded that of its maintainer line. The mean square values in Table (8) revealed that the variations between CMS lines, their maintainer lines and the two Egyptian cultivars were highly significant.

2.4. Flag leaf width:

Concerning the flag leaf width character, the maintainer lines IR68277B, and IR68276B, and the CMS lines IR68277A, and IR67701A, possessed the highest values of 1.530, 1.505, 1.505, and 1.500, respectively, followed by the maintainer line IR67701B, Giza181, the maintainer line IR68884B, the CMS line IR68276A, and Giza177 which had values of 1.490, 1.355, 1.32, 1.300, 1.225 cm, respectively (Table 7). On the other hand, IR68884A; the CMS line, showed the lowest mean value of 1.2 cm. Therefore, the differences between estimates for this character among the CMS lines, their maintainer lines, and the two Egyptian cultivars were highly significant (Table 8).

Table (7). Mean performance of the four CMS lines, their maintainer lines and the two Egyptian cultivars for the studied morphological and agronomic characters.

Characters / Genotypes	Plant height	No. of fertile tillers/ plant	Flag leaf length	Flag leaf width	Panicle length	Panicle excision	Heading date
a. CMS lines							
1. IR67701A	92.50± 1.5bc	11.55± 1.06c	25.71± 1.1bcd	1.500± 0.04a	18.06± 0.28bc	66.75± 2.05e	100.5± 0.22c
2. IR68276A	78.15± 1.5e	15.60± 0.95ab	20.88± 0.8f	1.300± 0.0bc	17.83± 0.27c	91.7± 1.79b	93.0 ± 0.35h
3. IR68277A	96.45± 0.8ab	15.70± 1.3ab	27.00± 0.9abc	1.505± 0.029a	18.95± 0.31bc	81.27± 1.72c	91.0 ±0 .36i
4. IR68884A	90.55± 0.6cd	11.90± 0.62 bc	24.63± 0.7cde	1.200± 0.03c	22.56± 0.32a	71.5± 1.6d	104.0± 0.2c
b.Maintainer lines							
1. IR67701B	93.10± 1.2 bc	12.60± 1.12abc	28.99± 0.8a	1.490 ± 0.04a	17.73± 0.28c	100± 0a	104.0± 0.22b
2. IR68276B	82.10± 1.99e	16.10 ±0.99a	21.52± 0.9ef	1.505± 0.056a	18.42± 0.29bc	100± 0a	97.0 ± 0.34e
3. IR68277B	99.15 ±0.8a	14.20± 1.05abc	26.03± 1abc	1.530± 0.02a	19.23± 0.28b	100± 0a	96.0± 0.3f
4. IR68884B	95.00± 0.86ab	15.10± 0.99abc	26.80± 0.8abc	1.320 ± 0.026bc	21.70± 0.43a	100 ± 0a	108.0± 0.32a
c. Local cultivar							
1. Giza177	86.80± 0.78d	16.10± 1.2a	22.67± 0.4def	1.225 ± 0.014bc	19.15± 0.18b	100.± 0a	95.0± 0.3g
2. Giza181	80.85± 1.2e	15.85 ±1.26a	28.50± 0.77ab	1.355± 0.03b	22.27± 0.58a	95.5 ± 1.08b	110.0 ± 0.35b
LS.D at p level 0.01	4.4297	3.9071	3.2038	1.2789	1.2896	4.3299	0.6091

Table (8). Analysis of variance and test of significance of genotypic differences between the ten genotypes for the studied morphological and agronomic characters.

Source	d.f.	M.S.						
		Plant Height	No. fertile tillers/ plant	Flag leaf length	Flag leaf width	Panicle length	Panicle excision	Heading date
Genotypes	9	1023.2**	64.680**	157.61 **	0.30319 **	69.871 **	3305.7 **	765.75**
Rep.	1	57.245	13.520	1.5665	0.06444	0.9800	16.531	302.27
Error	189	28.951	22.523	15.144	0.02413	2.4536	27.661	4.72

Significant at p level <0.01

2.5. Panicle length:

Mean estimates of this character for the CMS lines IR67701A, IR68276A, IR68277A, and IR68884A were 18.06,

17.83, 18.95, and 22.56 cm, respectively (Table 7). On the other hand, the maintainer lines IR67701B, IR68276B, IR68277B, and IR68884B possessed estimates of 17.73, 18.42, 19.23, and 21.70 cm, respectively. While, Giza177 and Giza181 had estimates of 19.15, and 22.27cm, respectively. Differences among all genotypes for this character were highly significant (Table 8).

2.6. Panicle excision:

All maintainer lines and Giza177; the Egyptian cultivar, showed the highest mean estimates for the panicle excision, which was 100 (Table 7). The CMS lines; IR67701A, IR68276A, IR68277A, and IR68884A, and Giza181; the Egyptian cultivar, had estimates of 66.75, 91.70, 81.27, 71.50, and 95.50, respectively. All estimates possessed highly significant differences (Table 8).

2.7. Heading date:

Mean estimates of heading date character for the CMS lines IR67701A, IR68276A, IR68277A, and IR68884A were100.5, 93.0, 91.0 and 104.0 days, respectively (Table 7). On the other hand, the maintainer lines IR67701B, IR68276B, IR68277B, and IR68884B possessed estimates of 105, 97, 96 and 108 days, respectively (Table 7). While, Giza177,and Giza181; the Egyptian cultivars, had estimates of 95 and 110 days, respectively. Therefore, the differences between estimates for this character among the CMS lines, their maintainer lines, and the two Egyptian cultivars were highly significant at 0.01 level of significance (Table 8).

All the morphological characters under investigation revealed highly significant variations among all the genotypes under study. In almost, the statistical analysis of all the morphological characters revealed that each maintainer line surmounted its CMS line for the characters of plant height, number of tillers per plant, flag leaf length, flag leaf width, panicle length and panicle excision. The CMS lines surmounted the maintainer lines in the heading date character. The local cultivars possessed medium values for all the characters under investigation except for panicle excision character where the local cultivars surmounted only the CMS lines, also the local cultivar Giza177 surmounted all the other maintainer lines in heading date character.

The results suggested that the morphological characters were influenced by the male sterility character. These reflections in

morphological characters may cause a reduction in yield components, which, was also reported by Xiaobang and Zebing (1989). The results also suggested that the CMS lines IR67701A and IR68276A should not be used in rice breeding program because they had negative effect for product tillers and panicle excision, which may affect the yield components. On their work using eight male sterile to study the influence on 12 agronomic characters of hybrid rice, Xiaobang and Zebing (1989) found that all eight lines had negative effects on eight different characters; plant height, neck length, panicles per plant, percentage of productive tillers, filled spikelets per panicle, filled spikelets percentage, 1000–grain weight, and grain weight per plant. With different combinations, they found that heading time was apparently delayed. They related the decrease in the yield of hybrid rice derived from A line was to the degradation of all yield components. Yield advantage of japonica rice was not found so large as indica hybrid rice because of narrow genetic base (Yuan, 1992).

The results from the molecular and morphological studies agreed well with those obtained from biochemical and cytological data (Megeed *et al.* in press). These results confirmed that the two CMS lines IR67701A and IR68276A displayed higher variations than the other CMS lines and these variations may cause a reduction in yield components.

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الاختلافات الجزيئية والوصفية لبعض السلالات ذات العقم الذكري السيتوبلازمى وسلالاتها المحافظة في الأرز

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دُرستْ الاختلافات الجزيئية والوصفية بين أربعة سلالات من الأرز ذات عقم ذكري سيتوبلازمي وأربعة سلالات محافظة وصنفين محليين علي مستوي الحامض النووي في الميتوكوندريا. وجد أن الحامض النووي الغير مهضوم يتكون من حزمة عريضة ذات وزن جزيئي كبير وهي المكون الرّئيسي وكذلك أربعة حزم منفصلة ذات وزن جزيئي ٢,١و١,٥و١,٦و٢,٣ كب ، أما الصنف المحلي جيزة١٨١ فقد أحتوي علي ثّلاثة حزم بأحجام ٢,١و١,٥و٢,٣ كب، واحتوي الصنف

جيزة ١٧٧ علي حزمتين بأحجام ١,٢ و ١,٥ كب. باستخدام ثلاثة إنزيمات قطع هي *SalI* و*KpnI* و*EcoRI* تم الحصول علي مجموع ٤٣ قطعة ، وتم الحصول علي خمسة طرز أحاديةhaplotypes باستخدام الإنزيم *SalI* ، وثمانية طرز أحادية باستخدام الإنزيم *KpnI* و٢٧ طراز أحادي باستخدام الإنزيم *EcoRI* . تراوح معامل الاستبدال النيكليوتيدي بين الطرز الأحادية المختلفة بين ٠,٠٠٧٨١±٠,٠٠٦٥ إلى ٠,١٢٥١±٠,٠٥٠٩ بينما كان هذا المعدل في السلالات ذات العقم الذكري السيتوبلازمي والسلالات المحافظة ٠,٠٠٣٩±٠,٠٠٣٣ ، وكان هذا المعدل بين السلالات تحت النوع الهندي والصنف تحت النوع الياباني ٠,٠٣٨٠±٠,٠٣٤٠ ، وكانت قيمة التباين النيكليوتيدي بين السلالات العقيمة ذكريا ٠,٠٠٥٢٧ وكانت بين السلالات المحافظة ٠,٠٠٤٣٨ ، وقدر الزمن منذ انفصال تحت النوع الهندي ونحن النوع الياباني بـ ٠,٠٢٢٤ مليون سنة مضت . تم بناء أشجار النسب الوراثية بين السلالات المختلفة باستخدام طريقة Neighbor-Joining. كما تمت دراسة سبعة صفات وصفية وهي طول النبات ،عدد الأفرع لكل نبات ، طول ورقةِ العلم، عرض ورقةِ العلم , طول السنبلة طرد السنبلة وعدد الأيام حتي التزهير. أوضحت كل الصفات الوصفية إختلافات هامّة إلى حدٍ كبير بين كل التركيب الوراثية . أوضح التحليل الإحصائي أن السلالات المحافظة فاقت مثيلاتها ذات العقم الذكري السيتوبلازمي وذلك بالنسبة للصفات طول النبات وعدد الأفرع لكل نبات ، طول ورقةِ العلم، عرض ورقةِ العلم , طول السنبلة ، طرد السنبلة. بينما كانت السلالات ذات العقم الذكري الستوبلازمي أعلي بالنسبة لصفة عدد الأيام حتي التزهير ، أظهرت الأصناف المحلية قيما وسطية لجميع الصفات فيما عدا صفة طرد السنبلة حيث كانت قيمها أعلي من قيم السلالات ذات العقم الذكري السيتوبلازمي بينما كانت قيم الصنف "جيزة١٧٧" أعلي من قيم جميع السلالات تحت الدراسة بالنسبة لصفة عدد الأيام حتي التزهير .