CYTOGENETICAL AND BIOCHEMICAL VARIATIONS OF SOME CYTOPLASMIC MALE STERILE LINES AND THEIR MAINTAINERS IN RICE

Mohammed S.A. Megeed, S.A. Dora, M.A.M. Nasser and M.A.W. Shehab

Genetics Department, Faculty of Agriculture, Tanta Univ., Kafr El-Sheikh, Egypt

ABSTRACT

Cytological and biochemical variations were studied to detect the variations between cytoplasmic male sterile (CMS) lines, their maintainer lines and two local cultivars of rice (*Oryza sativa*). The cytological behavior of the maintainer lines and local cultivars was normal and showed 12 bivalents. CMS lines were found to exhibit tetravalent (metaphase I), lagging chromosomes (anaphase I), and micronuclei (quartet). The presence of micronuclei in the CMS lines may be due to irregularity in the chromosomal behavior. The maintainer lines and the local cultivars revealed lower percentages of abortive pollen grains than that of the CMS lines, which showed percentages of sterility that ranged from 98.8% to 100%. This highly degree of sterility was due to the high percentage of chromosomal irregularities of the CMS lines, which, in turn, could reflect the role of the cytoplasm. The esterase and peroxidase analysis showed that there were differences between the CMS lines and maintainer lines in band number and band activity as well. The local cultivar Giza181 possessed the same number of bands as the CMS lines and maintainer lines. This result may be due to the fact that Giza181 is related to the Indica subspecies as the case of the CMS lines and their maintainer lines.

Key words:*Oryza sativa-CMS*-cytogenetical variation–biochemical variations.

INTRODICTION

Rice is one of the most important crops in the world, providing food for more than half the world's population. Compared to other cereal crops, rice has the smallest genome, $4.3x10^8$ bp (Arumuganathan and Earle, 1991) and is relatively easy to transform. The genetic syntony and microcolinearity between the rice genome

and other grass genomes has been well demonstrated (Ahn and Tanksley, 1993 and Park *et al.*, 2004).

The wild rice species offer a virtually untapped resource of agriculturally important genes that have the potential to solve many of the problems in rice production that we face today such as yield, drought tolerance, salt tolerance and disease and insect resistance (Vaughan *et al.*, 2003 and Kim *et al.*, 2005).

The rice genome has been one of the most intensively studied plant genomes during the last decade (Goff, 1999). The restriction fragment length polymorphism (RFLP) of rice was used extensively to acquire the construction of restriction fragment map (Harushima *et al.*, 1998 and Megeed *et al.*, 2004). In contrast to the rapid progress of molecular analyses of the rice genome, only limited success has been achieved toward a cytological characterization of the rice genome. Several laboratories have used fluorescence in situ hybridization (FISH) to map DNA sequences on rice chromosomes (Fukui *et al.*, 1994; Jiang *et al.*, 1995; Ohmido *et al.* 1998 and Cheng *et al.*, 2001). However, the majority of these reports involved mapping DNA sequences on mitotic metaphase chromosomes. Meiotic pachytene chromosome-based karyotypes have been previously reported, but none of these karyotypes are fully integrated with the currently available genetic linkage maps.

Hybrid rice seed production technology utilizes three different lines, namely a cytoplasmic male sterile line, CMS, (A line), a maintainer (B line), and a restorer (R line) (Virmani *et al.*, 2001). One of the conflicts of raising CMS lines is the failure to produce or release pollen grains due to the incompatibility between nuclear and mitochondrial genes. Rice hybrid must inherit a nuclear-based restoration of fertility system from the male parent so that the planted seeds are fully fertile.

This study aimed at understanding the cytogenetical and biochemical genetic variations among the CMS lines, their maintainer lines, and the Egyptian local cultivars.

MATERIALS AND METHODS

I-Plant Samples:

Ten different lines of rice (four CMS lines, four maintainer lines and two Egyptian cultivars) were used in this investigation (Table 1).

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
CMS lines		
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.
Maintainer lines		
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.
Egyptian Cultivars		
Giza177	Egypt (Giza171/ Yamji No.1 //pi. No 4	Japonica type, very early maturing, short grain, resistant to blast and high yielder.
Giza181	IRRI-Egypt (IR24 ×IR 22)	Indica type, medium maturing, excellent long grain and high yielder.

2- Cytological Studies:

Panicles before maturation were collected and kept in fixing and killing solutions which consists of three parts of absolute ethanol and one part of glacial acetic acid. Ferric chloride crystals (0.1% concentration) had been dissolved to give the fixed materials a fairly straw color. The materials were fixed at least 48h to as long as 20 days. After that, materials were hardened in 70% ethanol, washed three times in 70% ethanol and kept in 70% ethanol for immediately used or storing for as long as 30 days. For smear preparation, a single anther was squeezed in 1% acetocarmine stain. The slides were then pressed with several layers of filter papers and the clear-cut stages were tested for chromosomal counts and photographed using Reichert microscope.

3-Electrophoresis and isozyme techniques:

Two isozymes i.e., esterase and peroxidase were investigated in leaf extract obtained from 25 days old seedling. Both isozymes were determined employing the polyacrylamide gel slab of 7.5 % acrylamide for separation gel and 2.5 % acrylamide for stacking gel. These methods were outlined by Davis (1964). Two-tenth gram leaves tissues were homogenized in 1 mM of an ice-cold 50 mM tris-HCl buffer (pH 6.8) containing 20 % (w/v) sucrose and 3 mM dithiothritol (DTT). The extracts were then centrifuged at 15000 rpm at 4 °C for 5min. and supernatants were pipetted (Aly, 1986).

Esterase bands were detected as described by Scandalios (1969), by using alpha naphthyl acetate as substrate and subsequent color development with fast blue RR salts. Peroxidase bands were detected according to Tu *et al.* (1986) by using binzidine dihydrochloride as a substrate and subsequent color was developed with H_2O_2.

RESULTS AND DISCUSSION

1-Cytological Studies:

1.1-Chromosome associations at diakinesis and metaphase I stages:

Twelve bivalents were observed in each of the two Egyptian cultivars as well as in the four maintainer lines at diakinesis and metaphase I stages. Furthermore, no chromosomal aberrations in these six entities were detected at all phases. While, irregular chromosomal associations in the four CMS lines were found.

Data in Table (2) shows that at diakinesis stage, the CMS line IR68276A was found to possess the highest average number of quadrivalents and percentage of cells having either the form of eight-shaped quadrivalents or ring shaped quadrivalents plus ten bivalents, followed by the CMS line IR67701A. However, the other two CMS lines; IR68277A, and IR68884A showed the lowest estimates for such configurations (Figure 1a, b, and c). The number of the quadrivalents observed in the CMS lines, IR68276A, IR67701A, IR68884A and IR68277A ranged from zero to one, whereas the average numbers of quadrivalents for such CMS lines were found to be 0.25, 0.23, 0.21 and 0.20, respectively. Percent-

ages of meiocytes showing one quadrivalent plus ten bivalents in such CMS lines were recorded to be 25, 23, 21 and 20, respectively.

Table (2). The chromosome associations of the four CMS lines at diakinesis and metaphase 1 stages.

Genotype	Diakinesis					Metaphase I				
	No. of cells	Chromosome association				No. of cells	Chromosome association			
		I	II*	III	IV		I	II	III	IV
IR67701	77	-	12	-	-	90	-	12	-	-
	23	-	10	-	1	10	-	10	-	1
Total	100	-	1154	-	23	100		1180	-	10
Average	1.00	-	11.54	-	0.2	1.00	-	11.80	-	0.1
IR68276	75	-	12	-	-	88	-	12	-	-
	25	-	10	-	1	12	-	10	-	1
Total	100	-	1150	-	25	100	-	1176	-	12
Average	1.00	-	11.50	-	0.2	1.00	-	11.76	-	0.1
IR68277	80	-	12	-	-	91	-	12	-	-
	20	-	10	-	1	9	-	10	-	1
Total	100	-	1160	-	20	100	-	1182	-	9
Average	1.00	-	11.60	-	0.2	1.00	-	11.82	-	0.0
IR68884	79	-	12	-	-	89	-	12	-	-
	21	-	10	-	1	11	-	10	-	1
Total	100	-	1158	-	21	100	-	1178	-	11
Average	1.00	-	1158	-	0.2	1.00	-	11.78	-	0.1

*Total number of each unit of chromosome association=the observed number X no. of cells (those carrying that unit).

At metaphase 1 stage, the CMS line IR68276A exhibited the highest number of meiocytes having one quadrivalent plus ten bivalents with an average of 0.12 followed by IR68884A with an average of 0.11, while the two CMS lines; IR67701A and IR68277Awere found to have the lowest values; 0.10 and 0.09, respectively. Such obtained data revealed that the four CMS lines under study exhibited approximately similar frequencies of meiocytes having one tetravalents plus ten bivalents only as presented in Figure (1d, e and f). On the other hand, the percentages of meiocytes having one quadrivalents plus ten bivalents were recorded to be 12, 11, 10 and 9, respectively, for the CMS lines; IR68276A, IR68884A, IR67701A and IR68277A as shown in Table (2).

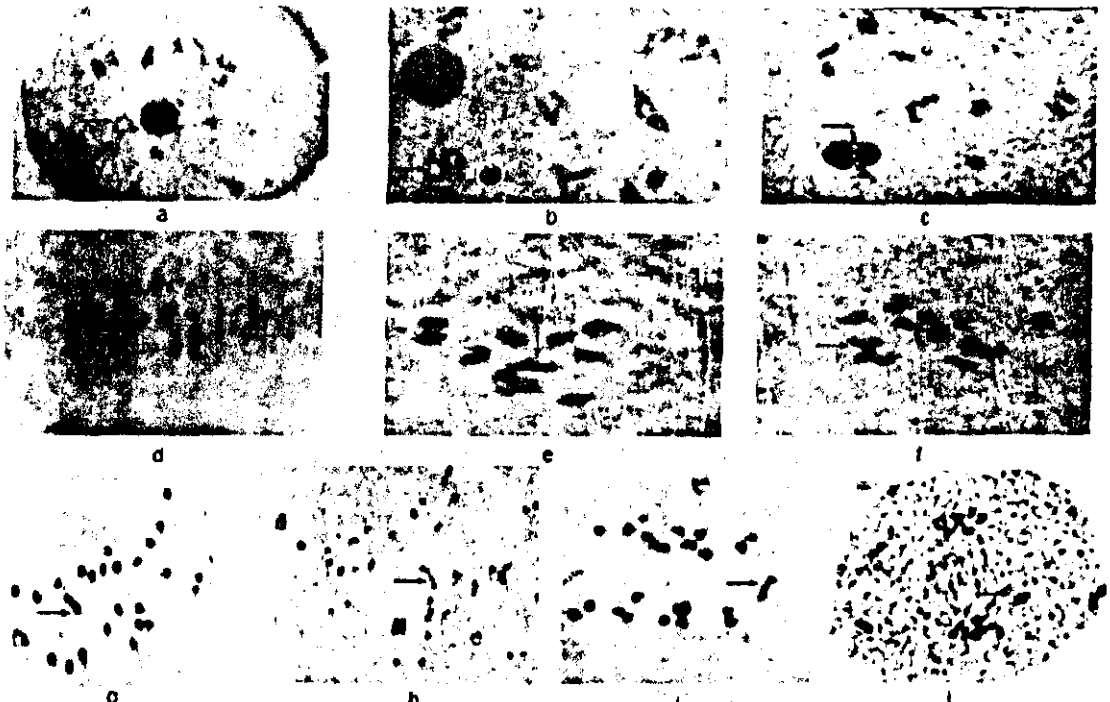

Figure 1. Photomicrographs (X=1500) of the diakinasis, metaphase I and anaphase I stages of the genotypes under study. (a) Diakinesis stage with one ring-shaped quadrivalent plus ten bivalents, (b) Diakinesis stage with one eight-shaped quadrivalent plus ten bivalents, (c) Diakinesis stage with one rod-shaped quadrivalent plus ten bivalents, (d) metaphase I stage showing one ring-shaped quadrivalent plus ten bivalents, (e)metaphaseIstage showing one rod-shaped quadrivalent plus ten bivalents, (f) metaphase I stage showing one Y-shaped quadrivalent plus ten bivalents, (g) early anaphase I shows one persistent chiasma – nunequal separation, h) anaphase I shows one persistent chiasma –nunequal distribution, (i) anaphase I shows two laggards, and (j) anaphase I shows one laggard.

1-2- Number of lagging chromosomes:

As shown in Table (3) at anaphase stage, the two CMS lines IR67701A, and IR68276A exhibited some laggards that ranged from one to two with an average of 0.22 and 0.25, respectively as shown in Figure (1 g, h, and j). However, the remaining two CMS lines; IR68277A and IR68884A possessed only one laggard with an average of 0.14 and 0.19, respectively.

At telophase I stage, the average numbers of laggards were found to be 0.18, 0.21, 0.11 and 0.14, respectively, in the CMS lines IR67701A, IR68276A, IR68277A and IR68884A (Figure (2a and b).

On the other hand, only two CMS lines; IR67701A and IR68276A possessed lagging chromosomes with an average of 0.14 and 0.16, respectively, at anaphase II stage. However, in the other two CMS lines, only one laggard was recorded with an average of 0.07 for IR68277A and 0.10 for IR68884A (Figure 2. c, d, and e).

At telophase II stage, lagging chromosomes were observed only in the CMS lines. In IR67701A, IR68276A, IR68277A, and IR68884A CMS lines, 90%, 89%, 95% and 92% of the telophase II cells showed no laggards, while the remaining percentages were counted to possess from one to two laggards with an average of 0.13, 0.14, 0.05, and 0.08, respectively, (Figure 2 f, g and h).

1-3- Number of micronuclei:

At quartet stage from one to two micronuclei were found in the CMS lines (Table 3). The average number of micronuclei in the CMS lines IR67701A and IR68276A were found to be 0.06, and 0.07, respectively. However, in the IR68277A, and IR68884A CMS lines, one micronuclei in their tetrad cells was observed with average of 0.02, and 0.04, respectively, (Figure 2i, j and k).

1-4-Number of microcytes:

At quartet stage, the four CMS lines showed only one microcyte with an average of 0.04, 0.05, 0.02 and 0.04, respectively, whereas, 96%, 95%, 98% and 96% of their tetrad cells showed no microcytes (Figure 2i, j and k).

1-5-Pollen grain viability:

Pollen grains were examined with respect to viability. Round and darkly stained pollen grains were considered viable and functional, however, the non -viable grains, non-functional, are the

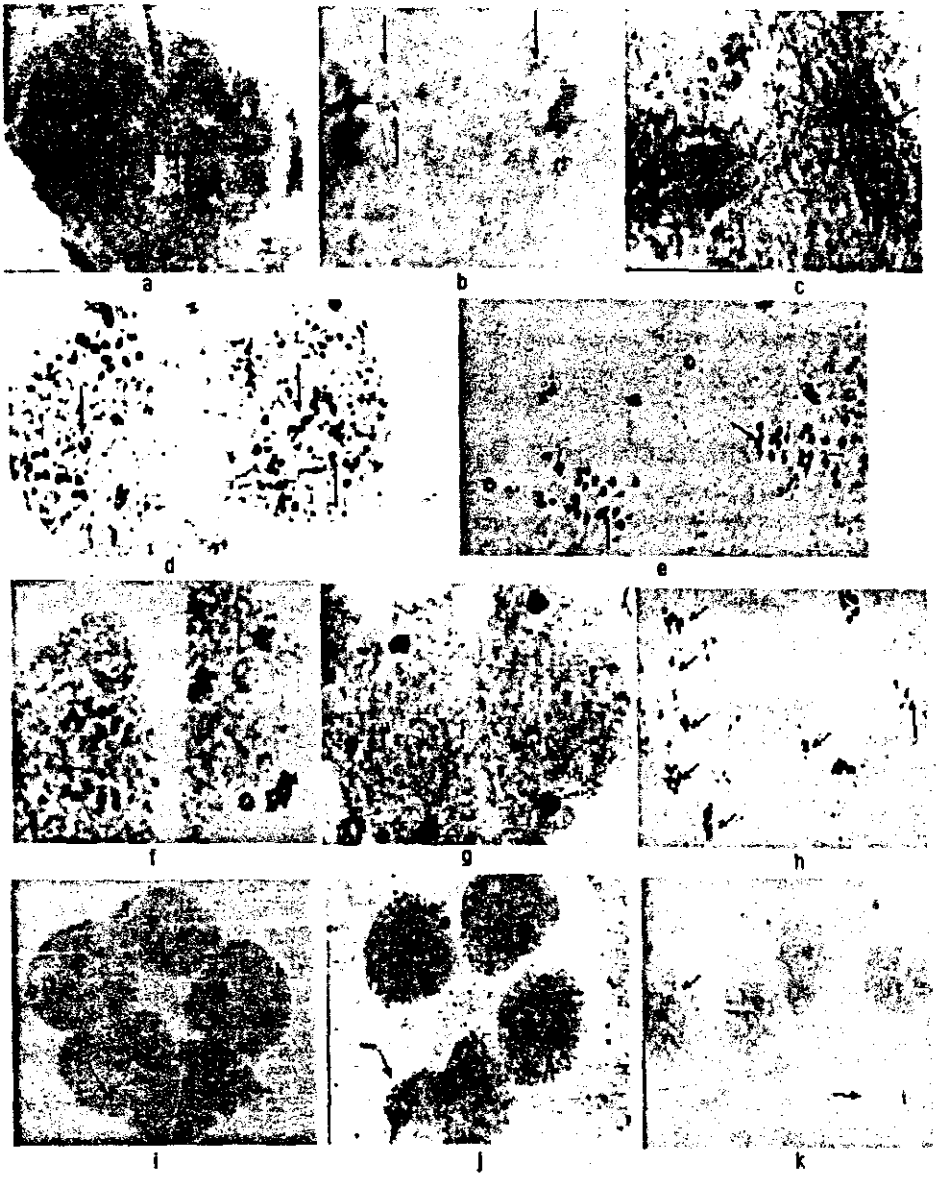

Figure2 Photomicrographs (X=1500) of the telophase I, anaphase II, telophase II and quartet stages of the genotypes under study. (a) telophase I stage with one laggard chromosome, (b) telophase I stage shows two laggards + one off pole, (c) anaphase II shows each cell takes perpendicular position plus one lagging chromosome in each one, (d) anaphase II shows each cell takes perpendicular position plus two laggards in each one, (e) anaphase II shows two laggards in each cell, (f) telophase II shows asynchronization, one still persists at anaphase II and the other shows tri-pole and laggarad at telophase, (g) telophase II shows bridge plus three laggards (h) telophase II shows tri-pole in one plus two laggards and the other cell shows three laggards, (i) quartet stage shows large microcyte and one of the tetrad cells has a micronuclei (j) quartet stage shows a microcye in abnormal fashion, and (k) quartet stage shows a microcyte and a micronuclei.

Table (3). Meiotic chromosome behavior at anaphase I, anaphase II, telophase I, telophase II, and quartet stage, number and average of lagging chromosome and micronuclei for CMS lines.

Genotype	Anaphase I		telophase I		anaphase II		telophase II		quartet			
	No. of cells	No. of lags	No. of cells	No. of lags	No. of cells	No. of lags	No. of cells	No. of lags	No. of tetrads	No. of micronuclei	No. of tetrads	No. of microcytes
IR67701A	84	-	87	-	89	-	90	-	96	-	96	-
	10	1	8	1	8	1	7	1	2	1	4	1
	6	2	5	2	3	2	3	2	2	2	-	-
Total	100	22	100	18	100	14	100	13	100	6	100	4
Average	1.00	0.22	1.00	0.18	1.00	0.14	1.00	0.13	1.00	0.06	1.00	0.04
IR68276A	82	-	85	-	89	-	89	-	95	-	95	-
	11	1	9	1	6	1	8	1	3	1	5	1
	7	2	6	2	5	2	3	2	2	2	-	-
Total	100	25	100	21	100	16	100	14	100	7	100	5
Average	1.00	0.25	1.00	0.21	1.00	0.16	1.00	0.14	1.00	0.07	1.00	0.05
IR68277A	86	-	89	-	93	-	95	-	98	-	98	-
	14	1	11	1	7	1	5	1	2	1	2	1
Total	100	14	100	11	100	7	100	5	100	2	100	2
Average	1.00	0.14	1.00	0.11	1.00	0.07	1.00	0.05	1.00	0.02	1.00	0.02
IR68884A	81	-	86	-	90	-	92	-	96	-	96	-
	19	1	14	1	10	1	8	1	4	1	4	1
Total	100	19	100	14	100	10	100	8	100	4	100	4
Average	1.00	0.19	1.00	0.14	1.00	0.10	1.00	0.08	1.00	0.04	1.00	0.04

shriveled, or lightly stained ones.

Table (4) presents the total numbers and percentages of the viable and aborted pollen grains for the four CMS lines, their maintainers, and the two Egyptian local cultivars. CMS lines IR67701A, and IR68276A recorded 100% percentage of non-viable pollen grains. In case of IR68277A and IR68884A; CMS lines, percentages of non-viable pollen grains were 98.8 and 99.1, respectively. On the contrary, the percentage of the non-viable pollen grains were 1.8, 2.2, 3.1, 2.5, 3.9, and 2.7, respectively.

Table (4). Total number and percentage of viable and abortive pollen grains of the four CMS lines, their maintainers and the two local cultivators.

Genotype	Number of pollen grain		Total	Non- viable %
	Viable	Non- viable		
CMS lines:				
1. IR67701A	0	1314	1314	100
2. IR68276A	0	2528	2528	100
3. IR68277A	24	1923	1947	98.8
4. IR68884A	18	1920	1938	99.1
Maintainer lines:				
1. IR67701B	1506	28	1534	1.8
2. IR68276B	1675	38	1713	2.2
3. IR68277B	1257	41	1298	3.1
4. IR68884B	1577	41	1618	2.5
Local cultivars;				
1. Giza177	1002	14	1016	3.9
2. Giza181	1238	35	1273	2.7

Concerning the cytological studies on the cytoplasmic male sterile of rice, no detailed research work has been reported on this subject. Laser and Lersten (1972) enumerated the crops on which the process of pollen abortion in male sterile lines were cytologically studied between 1925 and 1972; rice was not included. Shinjyo (1972) published a cytological study of pollen abortion in male sterile rice that same year. China began research on pollen abortion in male sterile rice in 1971, when breeding of CMS lines, maintainer lines and restorer lines was under way. By 1985, an estimated 36 male sterile lines of rice from 14 different cytoplasmic

sources had been studied (Yin and De- Sheng, 1985).

2-Electrophoresis of esterase and peroxidase isozymes:

In this study, an attempt was made to assess the variation of number and activity of the esterase and peroxidase isozyme patterns in the leaf crude extract of all rice lines under investigation.

Electrophoresis analysis for both esterase and peroxidase isozymes of the CMS lines showed variations in both number as well as activity of bands compared with their maintainer lines and the two local cultivars (Figure 3 and b, and Table 5). These results are reflecting the variation between the CMS lines, which may be due to the changes occurred in the genetic background of each one of them. These results were also true for the maintainers and the local cultivars. On the other hand, the variation between the CMS lines and their maintainers seems to be a reflection of the changes in the gene expression in these lines.

With respect to the esterase zymograms, all CMS lines, their maintainer lines, and the two local cultivars were found to be distributed in three zones (Table, 5 and Figure, 3a). The first zone, showed to possess two low intensity bands, bands no 1 and 2. In the second zone, however, there were five bands of numbers 3, 4, 5, 6, and 7, which were found to migrate faster. The bands no. 5 and 6 were higher in intensity than the other bands. On the other hand, band no. 7 was the lowest one. There were two bands observed in the third zone, no. 8, and 9, which were faster and their intensities were higher than that of all the other bands in the first or the second zone.

When comparing the activity and number of bands of the isozyme among the genotypes under study, it could be postulated that the highest activity in the first zone was detected for all the maintainer lines and the CMS line IR68276A, followed by the other two CMS lines; IR67701A, IR68277A, and the two local cultivars Giza181, and Giza177. In the second zone, however, the band no. 7 was detected only in the two local cultivars Giza181, and Giza177, and the maintainer line, IR67701B, while, it was absent in all the CMS lines and the rest of maintainers. All the maintainers and the local cultivar Giza181 showed the highest activity comparing to the CMS lines and the other local cultivar Giza177. In the third zone, however, Giza177; the local cultivar, and the

CMS line IR68884A exhibited only one band; (no.9), while all the other lines possessed two bands. The maintainer line IR67701B had the highest activity for band no.8 comparing to the other lines. The CMS lines and the local cultivars expressed lower activity than the maintainers did.

The analysis of peroxidase isozyme patterns, in the CMS lines, their maintainers and the two local cultivars, revealed that all these entries possessed nine to ten bands (Table 5, and Figure 3b), and they showed different degrees of activities. These peroxidase bands were distributed in four zones. In the first zone there were three bands; no 1, 2, and 3. All these three bands were observed in all the entries under investigation except for the CMS line, IR67701A, which had only two bands; no. 1 and 2. Also this CMS line showed the lowest activity for band no.2. There was also variation in activity for band no. 3, which showed lower activity in the other three CMS lines, IR68276A, IR68277A and IR68884A. Also in the second zone four bands were detected, no. 4, 5, 6 and 7. Each one of the studied genotypes was found to possess three bands with noticeable differences in their intensities. Band no 5 was only detected in the local cultivar Giza177 but it was absent in all the other lines. On the other hand, this local cultivar was the only line, which did not possess band no. 4. The CMS line IR67701A had the lowest activity for the other three bands; no 4, 6 and 7, which showed the highest activity only among the maintainer lines IR67701B, IR68276B, IR68277B and IR68884B. In the third zone, all of entries possessed two bands, no. 8, and 9, and their activities were different in each one of them. Again the maintainer lines and the local cultivar Giza181, expressed the highest activity for these two bands, (no 8 and 9). Also in the fourth zone, two bands were detected for all entries and they were numbered 10, and 11 with some differences in their activity. For these two bands, the maintainer lines and the two local cultivars showed the same trend while they had the higher activity.

Comparisons of either isozyme activity or number of bands considering all entries revealed that all of them were similar for the band no. 1, the same was true for the band no.2 except for IR67701A; the CMS line, that had the lowest intensity. On the other hand, band no. 3 was lost in IR67701A and all the rest of the

Table (5). Description of esterase and peroxidase isozyme patterns of the ten genotypes understudy.

Esterase										
Genotype	Giza181	Giza177	IR68884B	IR68884A	IR68277B	IR68277A	IR68276B	IR68277A	IR67701B	IR67701A
	Lane no.									
No. of bands	1	2	3	4	5	6	7	8	9	10
1	++	+	++++	+++	++++	+++	++++	++++	++++	+++
2	++	+	+	+	+	+	+	+	+	+
3	++	+	+++	+++	+++	+++	+++	+++	+++	+++
4	++	+	+	+	+	+	+	+	+	+
5	+++++	++++	+++++	++++	+++++	++++	+++++	++++	+++++	++++
6	+++++	++++	+++++	++++	++++	++++	+++++	++++	+++++	++++
7	+	+	—	—	—	—	—	—	+	—
8	+	—	+	—	+	+	+	+	+++	+
9	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++
Total	9	8	8	7	8	8	8	8	9	8
Peroxidase										
1	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++
2	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	++++
3	+++++	+++++	+++++	++++	+++++	++++	+++++	++++	+++++	—
4	+++	—	++++	+++	++++	+++	++++	+++	++++	++
5	—	+++	—	—	—	—	—	—	—	—
6	+++	+++	++++	+++	++++	+++	++++	+++	++++	++
7	++	++	++	++	++	++	++	++	++	++
8	+++++	++++	+++++	++++	+++++	++++	+++++	++++	+++++	++++
9	+++++	++++	+++++	++++	+++++	++++	++++	++++	+++++	++++
10	++++	++++	++++	+++	++++	+++	++++	+++	++++	+++
11	++++	++++	++++	+++	++++	+++	++++	+++	++++	+++
Total	10	10	10	10	10	10	10	10	10	9

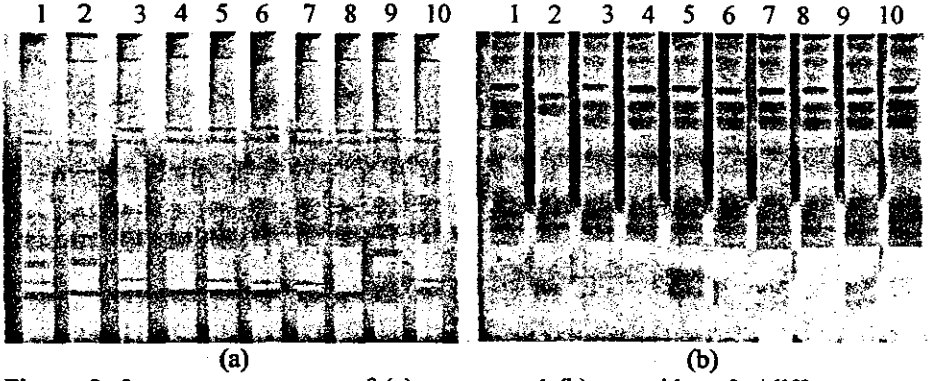

Figure 3. Isozyme zymogram of (a)esterase and (b) peroxidase for different genotypes under study. 1-Giza188, 2-Giza177, 3-IR68884B, 4- IR68884A, 5-IR68277B, 6-IR68277A, 7-IR68276B, 8- IR68276A, 9- IR67701B and 10-IR67701A.

CMS lines showed lower activity than the maintainer lines, which showed higher activity.

The band no. 5 was detected only in Giza177 and band no. 7 was similar in all the entries, however, the bands no. 4, and 6 showed the highest activity in maintainers and Giza181 than the CMS lines. Band no 4 was not detected in the local cultivar Giza177. In the third zone, all the CMS lines and Giza177 had the lowest activity comparing to the maintainers and the local cultivar Giza181. Meanwhile, in the fourth zone the maintainers and the local cultivars showed the highest intensities comparing to the CMS lines.

These results are in agreement with those obtained by Rongoian (1988), and Xiaobang and Zebing (1989) who suggested that there were differences in the activity and number of bands between CMS lines, maintainers and restorer lines. They suggested, also, that these variations might be a reflection of cytoplasmic effects on male sterile phenomenon.

Li *et al.* (1982) studied the relation between isozymes and heterosis in rice. They found that the hybrid combination whose F_1 had dominant complementary bands in the esterase isozyme was closely associated with the expression of heterosis. They added that, the zymogram of the F_1 of a strong hybrid combination is the complement of extraordinary enzyme bands of both parents.

The complementary enzyme bands may be used as one of the biochemical indicators for predicting heterosis. As the esterase isozyme of the F_1 has complementary enzyme bands, which differ

from the zymogram of its parents, this characteristic has been used in China to do preliminary evaluation of the purity of hybrid seeds. Yi *et al.* (1984) showed that both the cytoplasm and the nucleus of the parents influenced the zymogram pattern of the hybrid. Also they concluded that esterase isozyme patterns could be used as a biochemical marker for the predication of hybrid vigor in breeding.

A number of complementary high activity peroxidase enzyme bands was observed in the F_1 of some combinations with good heterosis (Zhou *et al.*, 1988 and Xiaobang and Zebing, 1989).

The observed isozyme variations between the CMS lines and their maintainers confirmed the using of zymograms variations as indicator for the CMS lines. The results obtained for the morphological characters (Megeed *et al.*, 2004) agreed well with those obtained from the isozyme variations, that the CMS lines are having lower estimates than all the other lines. Xiaobang and Zebing (1989) also derived these conclusions.

The results obtained previously by Megeed *et al.* (2004) and the data of the isozymes analysis as well as the cytogenetical analysis revealed that the CMS lines could be compared with, and also be distinguished from, their maintainers.

These results confirmed that the two CMS lines IR67701A and IR68276A had higher variations than the other CMS lines and these variations may cause a reduction in yield components (Megeed *et al.*, 2004).

REFERENCES

Ahn, S. and S.D. Tanksley. (1993). Comparative linkage maps of rice and maize genomes. *Proc. Natl. Acad. Sci.* **90:** 7980–7984.

Aly, A.A. (1986). Regulatory effects. thylakoid polypeptide composition in chloroplasts of sern gametophytes. Ph.D. Thesis, Hungary Academy of science.

Arumuganathan, K. and E.D. Earle. (1991). Nuclear DNA content of some important plant species. *Plant Mol. Biol. Rep.* **9**:208–218.

Cheng, Z.; C.R. Buell; R.A. Wing; M. Gu and J. Jiang. (2001) Toward a Cytological Characterization of the Rice Genome. *Genome Res., 11(12): 2133-2141.*

Davis, B.J. (1964). Disc electrophoresis. II. Method and application of serum proteins. Ann. N. Y. Acad. Sci. 121:404-427.

Fukui, K., N. Ohmido and G.S. Khush. (1994). Variability in rDNA loci in the genus *Oryza* detected through fluorescence in situ hybridization. *Theor. Appl. Genet.* **87:** 893–899.

Goff, S.A. (1999). Rice as a model for cereal genomes. *Curr. Opin. Plant Biol.* **2:** 86–89.

Harushima, Y.; M. Yano; A. Shomura; M. Sato; T. Shimano; Y. Kuboki; T. Yamamoto; S.Y. Lin; B.A. Antonio; A. Parco; H. Kajiva; N, Huang; k. Yamamoto; Y. Nagamura; N. Kurata; G.S. Khush and T. Sasaki.. (1998). A high-density rice genetic linkage map with 2275 markers using a single F_2 population. *Genetics* **148:** 479–494.

Jiang, J.; B.S. Gill; G.L. Wang; P.C. Ronald and D.C. Ward. (1995). Metaphase and interphase fluorescence in situ hybridization mapping of the rice genome with bacterial artificial chromosomes. *Proc. Natl. Acad. Sci.* **92:** 4487–4491.

Kim, H.R.; J.S.S. Ammiraju; Y. Yu; D. Kudrna; M. Luo; J.L. Goicoechea. W. Wang; W. Nelson; C. Soderlund; D. Brar; D.J. Mackill; B. Han; L. Stein; P. SanMiguel; S. Jackson and R. Wing. (2005). The *Oryza* map alignment project: The golden path to unlocking the genetic potential of wild rice species. Plant & Animal Genomes XIII Conference, January 15-19, 2005, San Diego, CA, USA.

Laser, K. D. and N. R. Lersten. 1972. Anatomy and cytology of microsporogenesis in cytoplasmic male sterile angiosperms. Bot. Rev. 3: 425-454.

Li, Z. B.; W.H. Shao; Y. K. Zhu; R. J. Li; Z. L. Liu and J. M. Wan. 1982. The study and practice of hybrid rice. Shanghai Acadimic and Technical Press, Shanghai, China.PP64.

Megeed, M.S.A.; S.A. Dora, M.A.M. Nasser and M.A.W. Shehab. (2004). Molecular and morphological variations of some cytoplasmic male sterile lines and their maintainers in rice. *J.Agric.Res.Tanta Univ.*, 30(3):676-704.

Ohmido, N.; Y. Akiyama and K. Fukui. (1998): Physical mapping of unique nucleotide sequences on identified rice chromosomes. *Plant Mol. Biol.*, 38, 1043-1052.

Park Y.I., A. Dixit, J.W. Yoo and J. Bennetzen. (2004). Further evidence of microcolinearity between barley and rice genomes at two orthologous regions. *Mol Cells*, 17(3):492-502.

Rongoian, J. 1988. Comparison of heterosis effects of different cytoplasm types in rice. Proceedings of the International Symposium on Hybrid Rice. Philippines. IRRI. P.259.

Scandalios, J.G. (1969). Genetic control of multiple molecular forms of enzymes in plants. *Bioch. Genet.* 3:37-79.

Shinjyo, C. 1972. Hybrid Rice III. Cyto-histological investigation of pollen degeneration in anther of cytoplasmic male sterile plants. *Jpn. J. Genet. 47:179-183.*

Tu, A.T.; J.A. Reinosa and Y.Y. Hsiao. (1986). Peroxidase activity of hemepeptides from hors heart cytochrom C. *Experintia* 24: 219-220.

Vaughan, D.A.; H. Morishima and K. Kadowaki. (2003). Diversity in the *Oryza* genus. *Curr. Opin. Plant. Biol.*, 2:139-46.

Virmani, S.S., C.X.Mao; R.S. Toledo; M. Hossain and A. Janaiah. (2001). Hybrid rice seed production technology and its impact on seed industries and rural employment opportunities in Asia. Paper presented at the International Workshop on Seed and Seedling Science and Technology, 10-15 June 2001, China.

Yi, Q.H.; S.Y. Shi and J. R. Jiang. 1984. Analysis of esterase isozyme in F_1 hybrids of *Oryza sativa* and their constituent three lines and their prediction of heterosis. *Acta. Botanica Sinica 26: 506-512.*

Yin,S.,R. and L. De-Sheng. 1985. Cytological studies on pollen abortion of Honglian 1024A of rice. Department of Biol. Hunan Normal Univ. China.

Xiaobang, S. and L. Zebing.1989. Genetic effects of cytoplasm on hybrid rice. Proceedings of the International Symposium on Hybrid Rice. Los Banos, Laguna (philippines). IRRI. 258-259.

Zhou, K.; H. Li and R. Li. 1988. Breeding male sterile lines by crossing indica varieties. Proceedings of the International Symposium on Hybrid Rice. Los Banos, Laguna (philippines). IRRI. P.2.

الاختلافات السيتووراثية والبيوكيميائية لبعض سلالات الارز ذات العقم الذكرى السيتوبلازمى وسلالاتها المحافظة عليها

محمد سيد عبد المجيد، سعيد عبد السلام درة، مصطفي عبد الرازق ناصر ومحمد شهاب
قسم الوراثة ، كلية الزراعة بكفر الشيخ ، جامعة طنطا ، مصر

الملخص العربي

أجرى هذا البحث لتحديد درجة الاختلافات السيتولوجية والبيوكيمائية بين ثمانية سلالات من الارز تابعة لتحت النوع الهندي أربعة منها كانت عقيمة الذكر سيتوبلازميا واربعة سلالات محافظة للسلالات ذات العقم الذكرى السيتوبلازمى وتضمنت الدراسة صنفين محليين احدهما تابع لتحت النوع الهندي والآخر تابع لتحت النوع الياباني . لوحظ أنه فى الدور التشتتى والاستوائي الأول للسلالات المحافظة والأصناف المحلية سالفة الذكر 12 وحدة ثنائية الكروموسومات ولم يلاحظ بها أي شذوذ كروموسومى ، بينما شوهدت فى السلالات ذات العقم الذكرى السيتوبلازمى شذوذات كروموسومية فى صوره رباعية الكروموسوم وكروموسومات متلكأه ووجود الأنوية الصغيرة وكذلك لوحظ وجود واحدة من الخلايا الصغيرة بنسب متقاربة فى الدور الرباعي ويرجع وجود هذه الانوية الصغيرة فى تلك السلالات إلى عدم الانتظام فى السلوك الكروموسومى فى الأدوار السابقة. ويحتمل أن تكون هذه الكروموسومات المتلكأه قد ظهرت نتيجة الانفصال المبكر للوحدات الكروموسومية فى الدور الاستوائي الأول مما يترتب عليه تكوين كروموسومات مفرده والتى يتلكأ بعضها أثناء الأدوار التالية. أفادت نتائج حيوية حبوب اللقاح فى الأصناف المحلية و السلالات المحافظة مرة أخرى السلوك الكروموسومى فى الأدوار الميوزية المختلفة السابقة لمرحلة تكوين الجاميطات. و من ثم فإن الأصناف المحلية و السلالات العقيمة قد أظهرت نسب منخفضة من عقم حبوب اللقاح عن تلك المحسوبة فى السلالات ذات العقم الذكرى السيتوبلازمى التى أظهرت من 98.8%إلى 100% عقم. لذلك فإن السلوك الكروموسومى فى الأدوار السابقة وكذلك نتيجة إلى تركيبها الوراثي و دور السيتوبلازم فإنها أظهرت هذه الدرجة العالية من العقم. وقد أشارت النتائج المتحصل عليها للتفريد الكهربي لإنزيمي الاسنيريز والبيوكسيديز إلي عدم وجود اختلاف فى عدد الحزم بين السلالات ذات العقم الذكرى السيتوبلازمى و السلالات المحافظة عليها إلا فى حالات قليلة جدا و لكن من الناحية الأخرى كان هناك اختلاف فى نشاط هذه الحزم بين السلالات المحافظة والسلالات ذات العقم الذكرى السيتوبلازمى و قد اظهر الاختبار أن الصنف المحلى جيزة181 اظهر نفس عدد الوحدات التى أظهرتها السلالات ذات العقم الذكرى السيتوبلازمى و السلالات المحافظة وهذا يرجع إلى أنة يقع تحت نفس النوع الذى تقع تحتة هذه السلالات و هو تحت النوع الهندي.