MOLECULAR POLYMORPHISM OF RICE CYTOPLASMIC GENETIC MALE STERILE (CMS), RESTORER LINES AND THEIR HYBRID COMBINATIONS BASED ON RAPD AND ISOZYMES ANALYSIS

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ABSTRACT

The cytoplasmic-genetic male sterility (CMS) system has been the cornerstone of hybrid breeding world-wide. Maintaining genetic diversity in CMS germplasm is essential for reducing genetic vulnerability and increasing the likelihood of developing heterotic combinations. Using CMS lines from different cytosterility sources gave a broad range of diversity in currently-used local hybrid rice germplasm. In this study, RAPD and isozyme markers were employed to study the genetic variation among four diverse CMS and three Egyptian restorer elite parental lines widely used in hybrid rice breeding programs in Egypt. However, the relationship of genetic variation with hybrid performance and heterosis was studied. Results of this study could be used to expedite the development of genetically diverse and heterotic rice hybrids in Egypt.

DNA from four diverse groups of rice plants selected (each comprising the male sterile, the restorer and F_1 hybrid) has been extracted and amplified by PCR with different 15 mer random DNA primers (RAPD analysis). Then, DNA has been analyzed by agarose gel electrophoresis and DNA bands scored as present or absent. Out of used five arbitrary 15 mer primers, four were identified and detected polymorphism. The primer Cat No. 9050309 (5 GTG GGA ATG TGG GCT 3) showed higher amplified DNA bands in the different genotypes. Two major amplified bands, < 2600 bp and ≃ 2100 bp appeared in the restorer parents, Giza 178 R and Giza 181 R while, the same band was absent in all CMS lines and Giza 182 R. One band of primer Cat No. 9050322 with 700 bp was detected only in the restorers parents Giza 178 R and Giza 182 R while the same band was absent in parent Giza 181 R.

Esterase and peroxidase isozymes analysis could be used as biochemical genetic marker among the parental lines and their F_1 hybrid combinations.

The bands No. 5, 6 and 7 for esterase and bands No. 1, 4, 5, 6 and 10 for peroxidase were showed different and varied from genotype to another for strength and larger width of bands. Band No. 7 was stained strongly and confirmed expression of esterase bands. Therefore, the results revealed that there are many types of bands indicators for heterosis in rice, the complementary band type is considered the most important.

INTRODUCTION

Exploitation of heterosis in F_1 hybrid producing by crossing genetically divergent pure lines is a well recognized tool to increase yield (Frankel, 1983). As successfully demonstrated in the People's Republic of China, hybrid rice technology appears to be a feasible and readily available option for raising the yield potential. In China, the area planted to hybrid rice is around 15 million hectares, which constitutes about 50% of the total rice area (Jirong, 2000). However, it is being adopted by more than 20 rice growing countries (Yuan, 2001).

CMS is widely used for hybrid rice breeding (Yuan and Virmani, 1988). Hybrid rice is developed by using three lines, cytoplasmic male sterile line, maintainer and restorer lines. Therefore, breeding of the three lines is laborious and time-consuming.

Prediction of heterosis is interesting to breeders of crops like rice and maize in which hybrids are commercially important. Screening combinations for superior F_1 performance and strong heterosis is the most costly and time-consuming process in hybrid rice breeding programs. If a simple, efficient, inexpensive and reliable method could be used to predict heterosis prior to expensive field testing, much of field work associated with making crosses and field evaluation would be eliminated and hybrid rice breeding programs would be accelerated (Xiao, 1996). Major advances have been made in DNA marker technology in rice during the last few years. A number of genes for resistance to diseases insects and other agronomic traits such as photoperiod sensitivity, wide compatibility, aroma and male sterility, have been tagged with DNA markers (Nguyen, 1998). Molecular markers are becoming useful in facilitating plant breeding. The identification of molecular markers that are closely linked to genes of agronomic importance represent an important step toward increasing the efficiency of selection in breeding (Zhang *et al.*, 1997). Recent studies have indicated that RAPD markers (random amplified polymorphic DNA) are a powerful technique to detect polymorphism in DNA sequence for analysis

and determining the genetic relationships among rice cultivars (Williams *et al.*, 1990). Markers are identified using arbitrary primers and allow the quick construction of genetic maps for any plant species or saturation of specific genomic regions with molecular markers (Martin *et al.*, 1991 and Micheli and Bova, 1997).

The polymerase chain reaction (PCR) provides a simpler, faster, safer and less expensive means for genome analysis compared with RFLPs. A single short oligonucleotide primer can amplify specific sequences of genomic DNA through PCR. Randomly amplified polymorphic DNA sequences (RAPDs) obtained by the use of random oligonucleotide primers in PCR have been extensively used as molecular markers for tagging genes (Borokova *et al.*, 1995; Chunwongse *et al.*, 1994; Martin *et al.*, 1991 and Ronald *et al.*, 1992). Saturating existing molecular maps (Giovannoni *et al.*, 1991), constructing molecular maps (Lefebvre *et al.*, 1995 and Link *et al.*, 1995).

Isoenzymes refers to multiple forms of a single enzyme, identical in properties and catalytic action, but different in molecular structure of protein. The enzyme is genetically controlled and is a bridge between genes and characters (Deng Hongde, 1988). Since Schwartz (1960) first used isoenzymes to study heterosis in maize, isoenzymes have been widely applied in genetics and biochemistry. Isoenzymes are considered useful biochemical markers in genetic and plant breeding research (Westphal and Wrick, 1989).

In the study presented here, RAPD and isozyme markers were employed to study the genetic variation among four diverse CMS and three Egyptian restorer elite parental lines widely used in hybrid rice breeding programs in Egypt. However, the relationship of genetic variation with hybrid performance and heterosis was studied.

MATERIALS AND METHODS

Materials :

1. Plant Materials :

The plant materials for the study comprised 12 hybrids obtained using line x tester fashion involving four diverse cytoplasmic male sterile (CMS) lines, i.e. IR 58025 A (Wild Abortive or WA Source) from IRRI, G 46 A (Gambiaca Source), Large Stigma A (Kalinga Source) from China

and IR 68885 A (Mutant Source) from IRRI as "Lines" and three Egyptian restorers, viz., Giza 178 R, Giza 181 R and Giza 182 R as "testers" (Table 1).

Table (1). Cytoplasmic male sterile (CMS) and restorers lines used for the experiment

Genotype	Cytoplasm source	origin
IR 58025 A	Wild Abortive (WA)	IRRI
G 46 A	Gambiaca	China
Large Stigma A	Kalinga	China
IR 68885 A	Mutant IR 62829 A	IRRI
Giza 178 R	Restorer	Egypt
Giza 181 R	Restorer	Egypt
Giza 182 R	Restorer	Egypt

Four sets of rice genotypes were used. Each set was composed by two pure lines (a male sterile, and restorer lines) and by the F_1 hybrid combination, as detailed in Table (2). All sterile lines were cytoplasmic-nuclear, i.e. with one genetic factor controlling male sterility in the cytoplasmic DNA and one or several pairs in the nucleus .

Table (2). Hybrid rice plants used for PCR and isozymes analysis

Plant group	CMS lines (1)	Hybrid combinations (2)	Restorer (3)	Hybrid combinations (4)	Restorer (5)	Hybrid combinations (6)	Restorer (7)
A	IR 58025 A	IR 58025 A/ Giza 178 R	G. 178 R	IR 58025 A/ Giza 181 R	G. 181R	IR 58025 A/ Giza 182 R	G. 182R
B	G 46 A	G 46 A/ Giza 178 R	"	G 46 A/ Giza 181 R	"	G 46 A/ Giza 182 R	"
C	Large St. A	Larga St. A/ Giza 178 R	"	Larga St. A/ Giza 181 R	"	Larga St. A/ Giza 182 R	"
D	IR 68885 A	IR 68885 A/ Giza 178 R	"	IR 68885 A/ Giza 181 R	"	IR 68885 A/ Giza 182 R	"

Lareg St. A = Large Stigma A

II. Buffers :

1. RAPD Analysis :

a) Isolation and purification DNA buffers :

All the buffers which used for isolation and purification were the same as in Sumbrook *et al.* (1989) and Van de Ven *et al.* (1990).

b) PCR reagents :

Ready to go PCR Beads (Amersham, Pharmaica Biotech. Cat. No. 27-9555-01) were used for PCR technique. Each tube contained all reagents except primer and template for performing 25 µl PCR amplification reaction.

c) Primers :

Five random primers 15-mer were used in this study provided by Gulf Biotech. laboratory. Table (3) listed the five random primers and their nucleotide sequences.

Table (3). Nucleotide sequences of the five random primers used in this study

No.	Cat. No.	Sequences
1	9050303	5 TGA GTG GTC TAC GTG 3
2	9050307	5 GTG GTT CTG AGG GAG 3
3	9050309	5 GTG GGA ATG TGG CCT 3
4	9050318	5 CCT GAC CCC CGT CAC 3
5	9050322	5 AAC CTC CCC CTG ACC 3

d) Electrophoresis and isozyme buffers :

Extraction buffers, tris borate buffer, monomer solution, gel preparation and gel developing were carried out according to El-Fadly *et al.* (1990) and Stegmann *et al.* (1989).

2. Methods :

I. DNA Isolation :

DNA samples were extracted from fresh young leaves harvested from four weeks old field plants according to Van de Ven *et al.* (1990). The purity and concentration of the isolated DNA were determined at 260 and 280 nm wave length values (using Ultraspec 1000 UV/Vis spectrophotometer) and calculated according to Sumbrook *et al.* (1989).

II. RAPD Technique :

Twenty ng from each purified genomic DNA and twelve ng of each random primer (15 mer) were applied to PCR beads and completed to 25 µl using sterile distilled water. The amplification protocol was carried out according to Williams *et al.* (1990) using Techne (Genius) Thermal Cycler as follows :

a) Denaturation at 95 °C for 5 min.
b) Thirty five cycle each consists of the following segments.
   1. Denaturation at 95 °C for one min.
   2. Primer annealing temperature calculated according to Qiagen (1997) and it was increased for 5 °C above Tm value to eliminate the mismatching and undesired amplified bands.
   3. Polymerization and extension at 72 °C for 3 min.
   4. Hold at 4 °C till analysis.

The PCR products were electrophorated (using Hoefer HE 99 x Max Submarine Electrophoresis unit) on 2% agarose and 1 x TBE buffer at consistent 100 Volt for about four hr.

The different bands size were determined against 100 bp ladder marker from Amersham Pharmcia Biotech Company. The separated bands were stained with ethedium promide and documented using both of Polaroid Instant Camera and UV Transeliminator.

III. Isozyme Analysis :

Electrophoresis was performed in vertical polyacrylamide gels as discontinuous buffer system as described by Iglesias *et al.* (1974). The detection techniques used for esterases and peroxidases were those reported by Glaszmann (1987).

RESULTS AND DISCUSSION

The selected rice genotypes of Table (2) are genetically homogeneous, i.e. that they do not show detectable genomic variation among seed of the same genotype. To verify this, DNA was extracted independently from leaflets of 10 individuals of each genotypes (the same method followed by Wang *et al.* (1994). In all cases, the RAPD fingerprints produced with different primers were found to be identical (data not shown). Therefore, successive analysis were performed routinely on DNA extracted from leaves pooled from at least ten seedling of each genotype.

For identifying informative oligonucleotide primers for the RAPD analysis, i.e. primers that could yield polymorphic bands in the fingerprints obtained with DNAs from the different rice genotypes selected belonging the four diverse sets or groups of hybrid rice genotypes, listed in Tables (1 and 2). Each included a male sterile line of diverse cytosterility source, restorer line and their F_1 hybrid.

Five random oligonucleotides were tested as primers in the presence of DNA from each genotype. A total of 44 different DNA bands was produced by PCR amplification. Amplified DNA bands varied in size from about 100 bp to < 2600 bp. Out of the 5 primers, four turned out to be informative, i.e., to give at least 4 polymorphic bands. The high number of co-migrating bands provided an internal control to monitor the reproducibility of the amplification patterns. On the other hand, the polymorphic ones provided a key to genotype identification.

Figure (1) show DNA fingerprints and the DNA polymorphisms obtained with 5 tested primers (9050303, 9050307, 9050309, 9050318 and 9050322). Table (4) summarizes the relevant results by listing 4 primers that produced polymorphic bands. Scoring of these as present (+) or absent (-) demonstrate that each of the tested rice genotype can be recognized by a unique banding pattern. Furthermore, bands from both parents are confirmed to be present in the fingerprint of the hybrid.

In this study the F_1 hybrids from crosses between four diverse CMS lines and three of Egyptian restorer lines were used to identify the molecular marker linked to the restorer genes (RG).

Out of 5 arbitrary oligonucleotides primers which used for screening polymorphism between the parental line of CMS and restorer lines, four primers were identified and detected polymorphism (Table 4 and Fig. 1).

Table (4). The 4 DNA random primers that produced the 23 polymorphic bands used for identifying each of the 28 tested rice accessions

DNA primer name	Sequence from 5 to 3	Number of bands in the gel		Size of polymorphic band (pb)	A							B							C							D						
		Total	Polymorphic		1	2	3	4	5	6	7	1	2	3	4	5	6	7	1	2	3	4	5	6	7	1	2	3	4	5	6	7
Gulf Biotech 9050303	5 TGA GTG GTC TAC GTG 3	7	4	2500	-	+	+	+	+	-	+	-	-	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+
				1500	-	+	+	-	-	+	+	-	-	+	+	-	+	+	-	+	+	+	-	+	+	+	+	+	-	+	+
				550	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
				500	+	+	+	+	-	-	-	+	+	+	+	-	+	-	+	+	+	+	-	+	-	+	+	+	+	-	+	-
Gulf Biotech 9050309	5 GTG GGA ATG TGG GCT 3	12	8	< 2600	-	-	+	-	+	-	-	-	-	+	+	+	-	-	-	-	+	+	+	+	-	-	-	+	+	+	+	-
				≥2100	-	-	+	-	+	+	+	-	-	+	-	+	-	-	-	-	+	-	+	-	-	-	+	+	-	+	-	-
				1800	-	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
				1700	-	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
				1100	-	+	+	-	+	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
				900	-	-	-	+	+	-	-	+	+	-	+	+	+	-	+	+	-	+	+	+	-	-	-	-	+	+	-	-
				500	-	-	-	+	+	+	-	-	-	-	+	+	-	-	-	+	-	+	+	-	-	-	+	-	-	+	-	-
				400	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+	+	-	-	-	-	-	-	+	-	-	-	-	-
Gulf Biotech 9050318	5 CCT GAC CCC CGT CAC 3	10	5	1200	+	+	+	+	-	-	-	+	+	+	+	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
				1000	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	+	-	+	-	+	-	+
				550	+	-	+	-	-	+	+	+	+	+	-	-	+	+	-	+	+	+	-	+	+	-	+	+	-	+	+	+
				500	-	-	+	-	+	-	-	+	-	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
				450	+	-	+	+	-	-	+	-	+	+	-	-	+	-	-	-	+	-	+	-	+	-	-	+	+	-	+	+
Gulf Biotech 9050322	5 AAC CTC CCC CTG ACC 3	8	6	2200	-	+	+	-	+	+	+	-	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	-	+	+	+
				2000	-	+	+	-	+	+	+	-	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	-	+	+	+
				1000	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
				700	+	-	+	+	-	+	+	+	+	+	+	-	+	+	+	+	+	-	-	+	+	+	+	+	+	-	+	+
				500	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
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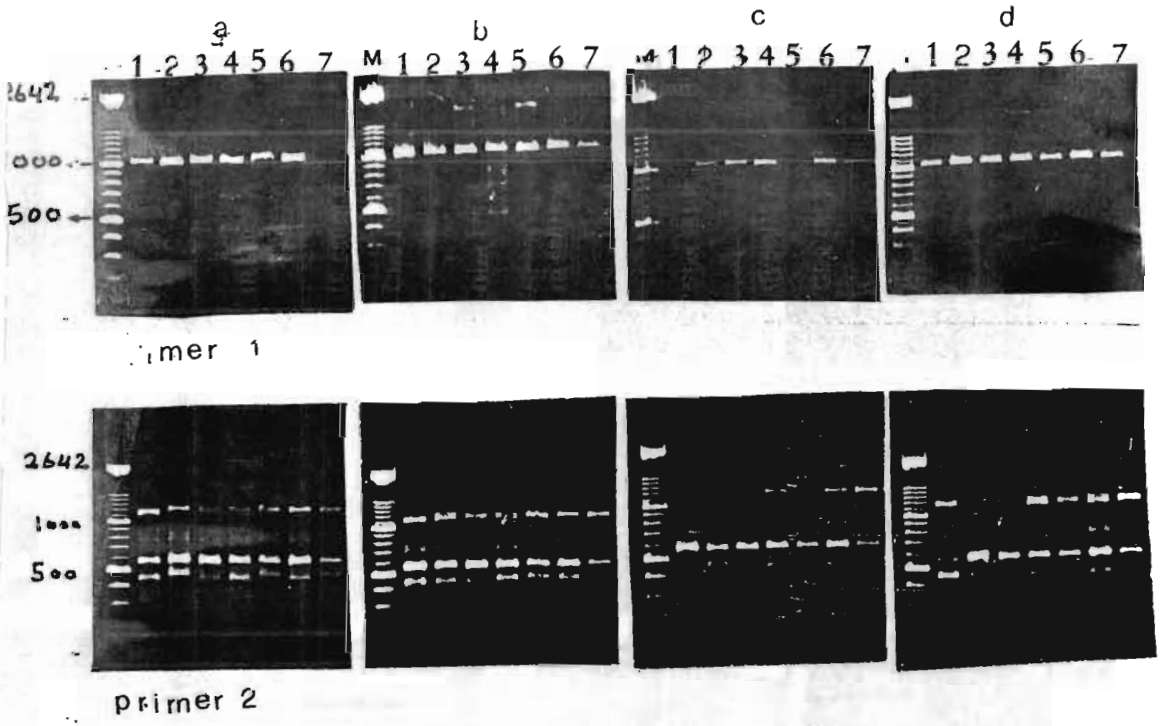

Fig. (1). Agarose gel electrophoresis of the DNA sequence obtained by PCR amplification of the DNA prepared from 4 different groups of rice plants (a, b, c and d) as a result of 5 random primers 15 mer.

M : 100 bp ladder marker

Plant group	Lane (1) CMS lines	Lane (2) Hybrid combinations	Lane (3) Restorer	Lane (4) Hybrid combinations	Lane (5) Restorer	Lane (6) Hybrid combinations	Lane (7) Restorer
a	IR 58025 A	IR 58025 A/ Giza 178 R	Giza 178R	IR 58025 A/ Giza 181 R	Giza 181R	IR 58025 A/ Giza 182 R	Giza 182R
b	G 46 A	G 46 A/ Giza 178 R	"	G 46 A/ Giza 181 R	"	G 46 A/ Giza 182 R	"
c	Large St. A	Larga St. A/ Giza 178 R	"	Larga St. A/ Giza 181 R	"	Larga St. A/ Giza 182 R	"
d	IR 68885 A	IR 68885 A/ Giza 178 R	"	IR 68885 A/ Giza 181 R	"	IR 68885 A/ Giza 182 R	"

Large St. A = Large Stigma A

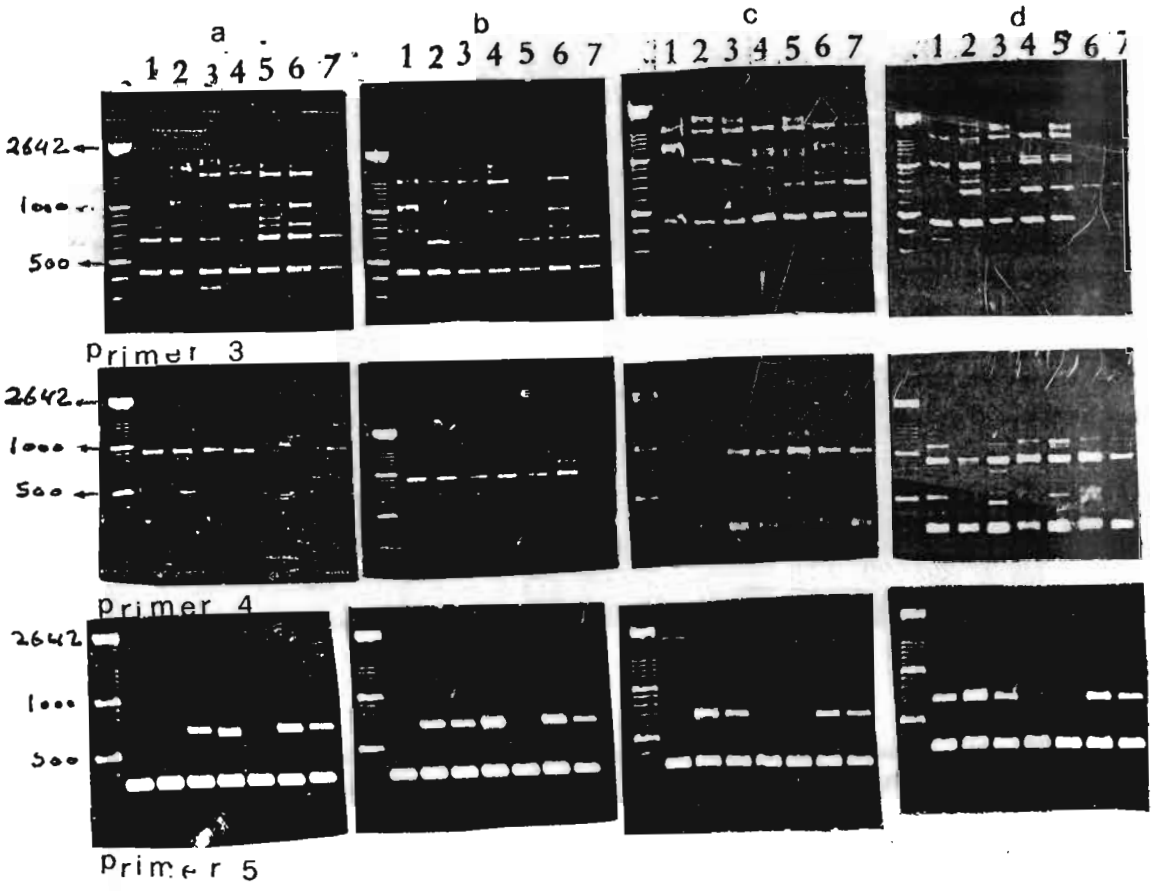

Fig. (1). Cont.

Plant group	Lane (1) CMS lines	Lane (2) Hybrid combinations	Lane (3) Restorer	Lane (4) Hybrid combinations	Lane (5) Restorer	Lane (6) Hybrid combinations	Lane (7) Restorer
a	IR 58025 A	IR 58025 A/ Giza 178 R	Giza 178R	IR 58025 A/ Giza 181 R	Giza 181R	IR 58025 A/ Giza 182 R	Giza 182R
b	G 46 A	G 46 A/ Giza 178 R	"	G 46 A/ Giza 181 R	"	G 46 A/ Giza 182 R	"
c	Large St. A	Larga St. A/ Giza 178 R	"	Larga St. A/ Giza 181 R	"	Larga St. A/ Giza 182 R	"
d	IR 68885 A	IR 58885 A/ Giza 178 R	"	IR 68885 A/ Giza 181 R	"	IR 68885 A/ Giza 182 R	"

Large St. A = Large Stigma A

On the other hand, the rest primer 9050318(5 CCT GAC CCC CGT CAC 3) showed monomorphic amplified bands since no differences could be detected (Figure 1 Primer 4).

Figure (1 Primer 3) showed higher amplified DNA in the different genotypes as a result of primer 9050309 (5 GTG GGA ATG TGG GCT 3) application. Since it amplified 12 bands with 8 polymorphic bands. It is clearly noticed that two major amplified bands, the first was < 2600 bp and the other was $\simeq$ 2100 bp appeared in the restorer parents Giza 178 R and Giza 181 R, but not in the restorer parent Giza 182 R and all CMS lines.

Figure (1 Primer 5) represents the resulted polymorphic bands from primer 9050322 (5 AAC CTC CCC CTG ACC 3) since it detected 5 polymorphic bands from 8 amplified bands. One band with 700 bp was detected in the restorer parent Giza 178 and Giza 182 but not in Giza 181.

In the present study, may be one or two fragment represented a single copy sequence obtained by using RAPD method and showed linkage with restore genes of Giza 178 R and Giza 181 R (primer 9050309) and Giza 178 R and Giza 182 R (primer 9050322).

Many studies have indicated RAPD analysis is powerful approach by which to find DNA polymorphism and screening differences between genomes (Xiao *et al.*, 1996; Young and Cheng, 1998; Ki *et al.*, 2000 and Reddy *et al.*, 2000).

The results showed that each of the rice genotype used for hybrid production and the F_1 hybrid itself can be identified by the selected RAPD markers. However, the RAPDs of the heterozygous F_1 hybrid genome always sum up the polymorphic bands of the homozygous parental lines. This offers a conventional tool for parentage assignation of F_1 hybrid.

The RAPDs may be used to quantify the most favourable genetic diversity among rice lines proposed for the production of heterosis. To this purpose date obtained with a number of primers are now routinely used to estimate the degree of dissimilarity among male sterile and restorer lines. The relationship between the dissimilarity value produced from RAPDs and true genetic distance among rice cultivars remains to be determined (Wang *et al.*, 1994). Yu and Nguyen (1994) have already shown that classification of rice cultivars based on RAPD analysis is identical to previous classification based on isozymes.

Isozyme analysis :

The present study as directed to identify if the esterase and peroxidase isozymes could be used as biochemical genetic marker among the four diverse cytoplasmic male sterile lines (CMS) i.e., (IR 58025 A, G 46 A, Large Stigma A and IR 6885 A), three Egyptian restorers, i.e., Giza 178 R, Giza 181 R and Giza 182 R and their F_1 hybrid combinations.

a) **Esterase :**

Results presented in Figures (2 a,b,c and d) and Tables (5,6,7 and 8) showed that the variation in number, migration and density of bands for esterase isozyme among the CMS, restorers and their F_1 hybrid combinations. The results showed that among four CMS lines were used, the G 46 A line exhibited seven bands as a maximum number of esterase isozyme bands. Since it gives two bands (No. 5 and No. 6) more than Large Stigma A and IR 68885 A lines and one (No. 6) more than IR 58025 A. Also, G 46 A line exhibited an intermediated band (No. 5) which not found in the rest of CMS lines.

The results indicated that also the three restorer lines exhibited six bands with different activity since Giza 181 R exhibited higher activity in the six band more than the other restorer lines.

Table (5). Description of esterase isozyme patterns of the CMS line IR 58025 A, the Egyptian restorers Giza 178 R, Giza 181 R and Giza 182 R and their F_1 hybrid combinations

Band No.	Genome type (A)						
	IR 58025 A	IR 58025 A x Giza 178 R	Giza 178 R	IR 58025 A x Giza 181 R	Giza 181 R	IR 58025 A x Giza 182 R	Giza 182 R
1	++	++	++	++	+++	++	+
2	+	+	+	+	+++	+	+
3	++	++	++	+	+++	+	++
4	++++	+++++	+++++	+++++	+++++	+++++	+++++
5	+++	+++	++	++++	+++	+++	++
6	-	-	-	-	-	-	-
7	++++	++++	+	++++	+++++	++++	+++
Total	6	6	6	6	6	6	6

- Absent + Very weak ++ Weak
+++ Intermediate ++++ Strong +++++ Very strong

Table (6). Description of esterase isozyme patterns of the CMS line G 46 A, the Egyptian restorers Giza 178 R, Giza 181 R and Giza 182 R and their F_1 hybrid combinations

Band No.	Genome type (B)						
	G 46 A	G 46 A x G. 178 R	G. 178 R	G 46 A x G. 181 R	G. 181 R	G 46 A x G. 182 R	G. 182 R
1	++	++	++	++	+++	++	+
2	+	+	+	+	+++	+	+
3	++	+++	++	++	+++	++	++
4	+++++	+++++	+++++	+++++	+++++	+++++	+++++
5	+++	+++	++	++	+++	++	++
6	+++	++	-	+++	-	+++	-
7	++++	++++	+	++++	+++++	+++	+++
Total	7	7	6	7	6	7	6

- Absent + Very weak ++ Weak
+++ Intermediate ++++ Strong +++++ Very strong

Table (7). Description of esterase isozyme patterns of the CMS line Large Stigma A, the Egyptian restorers Giza 178 R, Giza 181 R and Giza 182 R and their F_1 hybrid combinations

Band No.	Genome type (C)						
	Large Stigma A	Large Stigma A x Giza 178 R	Giza 178 R	Large Stigma A x Giza 181 R	Giza 181 R	Large Stigma A x Giza 182 R	Giza 182 R
1	+	+	++	+	+++	++	+
2	+	+	+	+	+++	+	+
3	++	+	++	-	+++	+	++
4	+++++	+++++	+++++	+++	+++++	+++++	+++++
5	-	-	++	-	+++	+++	++
6	-	-	-	-	-	-	-
7	++++	++++	+	+++	+++++	+++	+++
Total	5	5	6	4	6	6	6

- Absent + Very weak ++ Weak
+++ Intermediate ++++ Strong +++++ Very strong

Table (8). Description of esterase isozyme patterns of the CMS line IR 68885 A, the Egyptian restorers Giza 178 R, Giza 181 R and Giza 182 R and their F_1 hybrid combinations

Band No.	Genome type (D)						
	IR 68885 A	IR 68885 A x Giza 178 R	Giza 178 R	IR 68885 A x Giza 181 R	Giza 181 R	IR 68885 A x Giza 182 R	Giza 182 R
1	+	+	++	+	+++	+	+
2	+	+	+	+	+++	+	+
3	+	++	++	++	+++	++	++
4	++++	+++++	+++++	+++++	+++++	+++++	+++++
5	-	-	++	-	+++	-	++
6	-	-	-	-	-	-	-
7	++++	+++++	+	+++++	+++++	++++	+++
Total	**5**	**5**	**6**	**5**	**6**	**5**	**6**

- Absent + Very weak ++ Weak
+++ Intermediate ++++ Strong +++++ Very strong

Figure (2-a) and Table (5) show the esterase isozyme patterns and the degree of activities for each F1 hybrid combination descendant from the crosses between CMS line IR 58025 A and three restorer lines (Giza 178 R, Giza 181 R and Giza 182 R). These results revealed that all of the three hybrids exhibited six bands like their parents. On the other hand, the activities of the hybrid bands were similar to the female or CMS line with the exception band No. 7 on both hybrids (IR 58025 A/Giza 178 R and IR 58025 A/Giza 182 R) and the band No. 5 in the hybrid (IR 58025 A/ Giza 181 R) which presented high activity.

Figure (2-b) and Table (6) also showed that all of the three hybrid combinations resulted from the CMS line G 46 A with the three Egyptian restorers, Giza 178 R, Giza 181 R and Giza 182 R exhibited one band more than the restorer lines but like CMS line G 46 A, this band No. 6 was the same activity as well as most bands in the hybrids.

Figure (2-c) and Table (7) illustrate esterase isozyme patterns and their intensity for large stigma A line and the three restorers and their F_1 hybrid combinations. The data indicated that all crosses were lower than their parents.

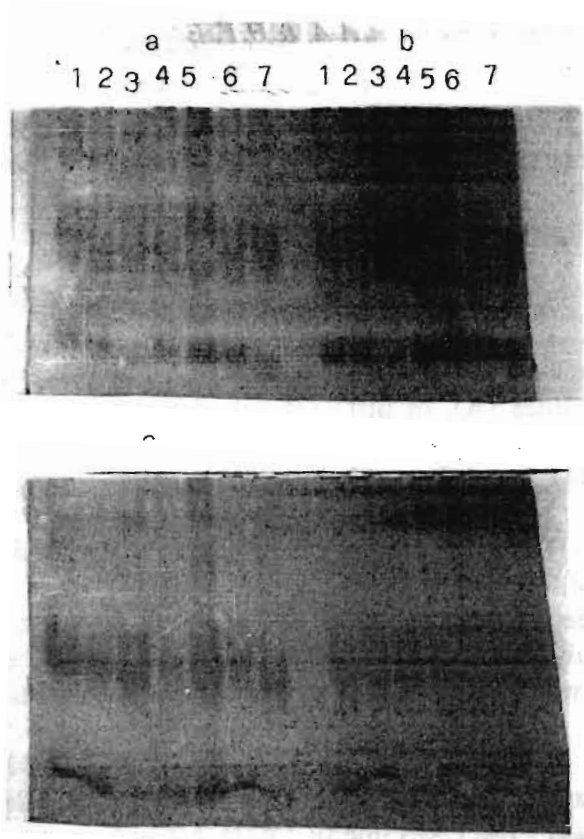

Fig. (2). Electrophoretic banding patterns of 4 different groups of rice plants (a, b, c and d) esterase isozymes.

Plant group	Lane (1) CMS lines	Lane (2) Hybrid combinations	Lane (3) Restorer	Lane (4) Hybrid combinations	Lane (5) Restorer	Lane (6) Hybrid combinations	Lane (7) Restorer
a	IR 58025 A	IR 58025 A/ Giza 178 R	G. 178 R	IR 58025 A/ Giza 181 R	G. 181 R	IR 58025 A/ Giza 182 R	G. 182 R
b	G 46 A	G 46 A/ Giza 178 R	"	G 46 A/ Giza 181 R	"	G 46 A/ Giza 182 R	"
c	Large St. A	Larga St. A/ Giza 178 R	"	Larga St. A/ Giza 181 R	"	Larga St. A/ Giza 182 R	"
d	IR 68885 A	IR 68885 A/ Giza 178 R	"	IR 68885 A/ Giza 181 R	"	IR 68885 A/ Giza 182 R	"

Large St. A = Large Stigma A

Figure (2-d) and Table (8) showed also that all the three hybrid combinations, IR 68885 A/Giza 178 R, IR 68885 A/Giza 181 R and IR 68885 A/Giza 182 R were exhibited the same number of bands like the CMS line with a higher activity in bands No. 3 and 4.

Esterase isozymes of hybrid materials have been analyzed in the leaves of seedlings. This study aims to identify specific hybrids for heterosis breeding by isozyme analysis. The leaves were sampled from male sterile lines (A), of different cytoplasmic sources, IR 58025 A, G 46 A, Large Stigma A and IR 68885 A, Egyptian restorers lines Giza 178 R, Giza 181 R and Giza 182 R and their hybrid combinations.

Polyacrylamid gel electrophoresis was used for esterase analysis. A total of 7 bands were found and numbered from 1 A to 7 A according to their decreasing mobilities (Table 9). Of these, 5 A, 6 A and 7 A bands showed different and varied from genotype to another for strength and larger width of bands, and were called "characteristic bands".

Observations of the three abovementioned bands in the cytoplasmic male sterile lines (CMS), restorer lines (R) and their hybrids are shown in Table (9). The CMS line IR 58025 A had two of the three bands, two CMS lines namely, Large Stigma A and IR 68885 A had one of the three bands and G 46 A had all the three bands. All the hybrids obtained from G 46 A with the three restorers Giza 178 R, Giza 181 R and Giza 182 R (G 46 A/Giza 178 R, G 46 A/Giza 181 R and G 46 A/Giza 182 R) also showed the three bands. However, the hybrids of IR 58025 A (with all the three restorers lines (IR 58025 A/Giza 178 R, IR 58025 A/Giza 181 R and IR 58025 A/Giza 182 R) had two of the three bands. The hybrids IR 58025 A/Giza 178 R, G 46 A/Giza 178 R, IR 58025 A/Giza 181 R and G 46 A/Giza 182 R performed well under field conditions and were found promising ones with yield advantage of 2.243, 2.725, 2.850 and 2.950 t/ha, respectively. However these hybrids showed also expression of esterase bands.

Table (9) shows all the hybrids with 7 A band only were stained strongly and confirmed expression of esterase bands. Cytoplasmic identifications of hybrids is difficult because of small chromosome size, and identification by morphological characters is sometimes unreliable . The Est isozyme sampled from leaves are stable and useful for identification (Liu *et al.*, 1992).

Table (9). Identification of CMS, restorers, hybrid combinations and expression of esterase bands 5A, 6A and 7A from leaves of three week old seedling

Materials	Cross	5 A	6 A	7 A	SH % for yield
IR 58025 A	Female	+++	-	+++	
G 46 A	Female	+++	+++	++++	
Large Stigma A	Female	-	-	++++	
IR 68885 A	Female	-	-	+++++	
IR 58025 A/Giza 178 R	F_1	+++	-	++++	21.63**
G 46 A/Giza 178 R	F_1	+++	++	++++	25.71**
Large Stigma A/Giza 178 R	F_1	-	-	++++	
IR 68885 A/Giza 178 R	F_1	-	-	+++++	12.74**
Giza 178 R	Male	++	-	+	
IR 58025 A/Giza 181 R	F_1	++++	-	++++	26.89**
G 46 A/Giza 181 R	F_1	++	+++	++++	6.84
Large Stigma A/Giza 181 R	F_1	-	-	+++	-26.85**
IR 68885 A/Giza 181 R	F_1	-	-	+++++	20.28**
Giza 181 R	Male	+++	-	+++++	
IR 58025 A/Giza 182 R	F_1	+++	-	++++	-12.73*
G 46 A/Giza 182 R	F_1	++	+++	++++	27.83**
Large Stigma A/Giza 182 R	F_1	+++	-	++++	-10.75
IR 68885 A/Giza 182 R	F_1	-	-	++++	-21.22**
Giza 182 R	Male	++	-	+++	

- Absent + Very weak ++ Weak
+++ Intermediate ++++ Strong +++++ Very strong
** Best hybrid combinations with high standard heterosis for grain yield

b) <u>Peroxidase</u> :

Variation in number and activity of bands are shown in Figures (3a, 3b, 3c and 3d) and Tables (10, 11, 12, 13 and 14) for the peroxidase. The results showed that sub-patterns were observed among the different tested genotypes. These sub-patterns have specific bands which were proven to may be associated with maternal effect of cytoplasmic male sterile line, paternal effect of restorer line and also may be the heterosis effect of hybrid combinations, but these differ from genotype to another under this study. Six bands (distributed in two groups, slow and fast mobility) were detected in the tables for two CMS lines, IR 58025 A (WA), Large Stigma A (Kalinga), four bands for G 46 A (Gambiaca) and seven bands for IR 68885 A (mutant).

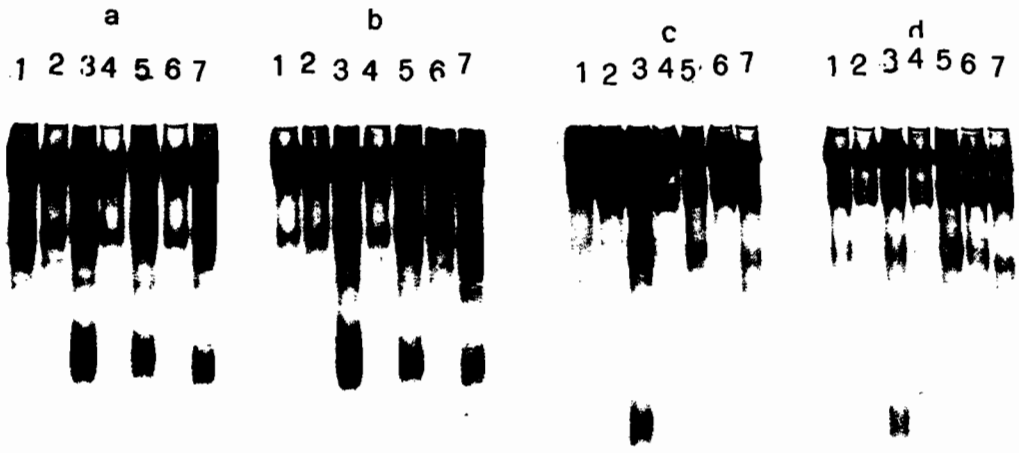

Fig. (3). Electrophoretic banding patterns of 4 different groups of rice plants (a, b, c and d) peroxidase isozymes.

Plant group	Lane (1) CMS lines	Lane (2) Hybrid combinations	Lane (3) Restorer	Lane (4) Hybrid combinations	Lane (5) Restorer	Lane (6) Hybrid combinations	Lane (7) Restorer
a	IR 58025 A	IR 58025 A/ Giza 178 R	G. 178 R	IR 58025 A/ Giza 181 R	G. 181 R	IR 58025 A/ Giza 182 R	G. 182 R
b	G 46 A	G 46 A/ Giza 178 R	"	G 46 A/ Giza 181 R	"	G 46 A/ Giza 182 R	"
c	Large St. A	Larga St. A/ Giza 178 R	"	Larga St. A/ Giza 181 R	"	Larga St. A/ Giza 182 R	"
d	IR 68885 A	IR 68885 A/ Giza 178 R	"	IR 68885 A/ Giza 181 R	"	IR 68885 A/ Giza 182 R	"

Large St. A. = Large Stigma A

Many scientists have studied the relation between isozymes and heterosis in rice. They found that the hybrid combination whose F_1 dominant complementary bands in the esterase isozymes was closely associated with expression of heterosis (Li *et al.*, 1982). That is, the enzymogram of the F_1 of a strong hybrid combination is predominantly the complemented 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 (Deng Hondge, 1988). Yi (1981) showed that biological heterosis and economic heterosis are caused by the respective complementary bands at different loci (the former is complement of 3A with 6A, and the latter is a complement of 5A with 6A). Many scientists studied also the correlation of the peroxidase isoenzyme with heterosis in hybrid rice. In the F_1 of some combinations with good heterosis a number of complementary enzyme bands with high activity were observed. Meanwhile, new bands were found in the F_1 of other combinations with good heterosis. They considered this phenomenon as another zymogram indicator (besides the complementary bands) for expression of heterosis (Li *et al.*, 1982).

From this study the relation between the isoenzyme of hybrid rice and heterosis show that complementation is an important biochemical indicator for heterosis, but not all combinations of complementary bands type exhibit heterosis (Tables 9 and 14). Heterosis is also expressed in the combinations of noncomplementary band type. The results revealed that there are many types of band indicators for heterosis in rice complementary band type, dominant band type, heterozygous band type, and distinctive band types (Tables from 5 to 14) and Figs. (2 and 3). The complementary band type, is considered the most important (Deng and Wang, 1984).

Table (10). Description of peroxidase isozyme patterns of the CMS line IR 58025 A, the Egyptian restorers Giza 178 R, Giza 181 R and Giza 182 R and their F_1 hybrid combinations

Band No.	Genome type (A)						
	IR 58025 A	IR 58025 A x Giza 178 R	Giza 178 R	IR 58025 A x Giza 181 R	Giza 181 R	IR 58025 A x Giza 182 R	Giza 182 R
1	-	-	+++	-	-	-	+++
2	+++++	++++	+++++	++++	+++++	++++	+++++
3	+++++	++++	+++++	++++	+++++	++++	+++++
4	-	-	++++	-	++++	-	-
5	+++	+++	-	+++	-	+++	+++
6	+	+	++	-	++	-	++
7	+	+	++	-	+	-	+
8	-	-	+++	-	++	-	++
9	++	++	++++	+	+++	++	+++
10	-	-	+	-	-	-	-
Total	6	6	9	4	7	4	8

- Absent + Very weak ++ Weak
+++ Intermediate ++++ Strong +++++ Very strong

Table (11). Description of peroxidase isozyme patterns of the CMS line G 46 A, the Egyptian restorers Giza 178 R, Giza 181 R and Giza 182 R and their F_1 hybrid combinations

Band No.	Genome type (B)						
	G 46 A	G 46 A x Giza 178 R	Giza 178 R	G 46 A x Giza 181 R	Giza 181 R	G 46 A x Giza 182 R	Giza 182 R
1	-	-	+++	-	-	-	+++
2	++++	++++	+++++	++++	+++++	+++++	++++
3	++++	++++	+++++	++++	+++++	+++++	+++++
4	-	-	++++	-	++++	+++	-
5	++	++	-	++	-	-	+++
6	-	-	++	-	++	++	++
7	-	-	++	-	+	+	+
8	-	-	+++	-	++	+	++
9	++	++	++++	+	+++	++	+++
10	-	+	+	-	-	+	-
Total	4	5	9	4	7	8	8

- Absent + Very weak ++ Weak
+++ Intermediate ++++ Strong +++++ Very strong

Table (12). Description of peroxidase isozyme patterns of the CMS line Large Stigma A, the Egyptian restorers Giza 178 R, Giza 181 R and Giza 182 R and their F_1 hybrid combinations

Band No.	Genome type (C)						
	Large Stigma A	Large Stigma A x Giza 178 R	Giza 178 R	Large Stigma A x Giza 181 R	Giza 181 R	Large Stigma A x Giza 182 R	Giza 182 R
1	-	-	+++	-	-	-	+++
2	++++	++++	+++++	++++	+++++	++++	+++++
3	++++	++++	+++++	++++	+++++	++++	+++++
4	++	++	++++	++	++++	+	-
5	+	+	-	+	-	-	+++
6	-	-	++	-	++	-	++
7	-	-	++	-	+	-	+
8	+	+	+++	-	++	+	++
9	++	++	++++	+	+++	+	+++
10	-	-	+	-	-	-	-
Total	6	6	9	5	7	5	8

- Absent + Very weak ++ Weak
+++ Intermediate ++++ Strong +++++ Very strong

Table (13). Description of peroxidase isozyme patterns of the CMS line IR 68885 A, the Egyptian restorers Giza 178 R, Giza 181 R and Giza 182 R and their F_1 hybrid combinations

Band No.	Genome type (D)						
	IR 68885 A	IR 68885 A x Giza 178 R	Giza 178 R	IR 68885 A x Giza 181 R	Giza 181 R	IR 68885 A x Giza 182 R	Giza 182 R
1	-	-	+++	-	-	-	+++
2	+++++	++++	+++++	+++++	+++++	++++	+++++
3	++++	++++	+++++	++++	+++++	++++	+++++
4	+++	+++	++++	+++	++++	+++	-
5	-	-	-	-	-	-	+++
6	++	++	++	+	++	++	++
7	+	+	++	+	+	+	+
8	++	++	+++	+	++	++	++
9	++	++	++++	++	+++	++	+++
10	-	+	+	-	-	-	-
Total	7	8	9	7	7	7	8

- Absent + Very weak ++ Weak
+++ Intermediate ++++ Strong +++++ Very strong

Table (14). Identification of CMS (female), restorers (male), hybrid combinations and expression of peroxidase bands IA, 4A, 5A, 6A and 10A from leaves of three week old seedling

Materials	Cross	1 A	4 A	5 A	6 A	10 A	SH% over best local check**
IR 58025 A	Female	-	-	+++	+	-	-
G 46 A	Female	-	-	++	-	-	-
Large Stigma A	Female	-	++	+	-	-	-
IR 68885 A	Female	-	+++	-	++	-	-
IR 58025 A/Giza 178 R	F_1	-	-	+++	+	-	21.63**
G 46 A/Giza 178 R	F_1	-	-	++	-	+	25.71**
Large Stigma A/Giza 178 R	F_1	-	++	+	-	-	0.00
IR 68885 A/Giza 178 R	F_1	-	+++	-	++	+	12.74**
Giza 178 R	Male	+++	++++	-	++	+	-
IR 58025 A/Giza 181 R	F_1	-	-	+++	-	-	26.89**
G 46 A/Giza 181 R	F_1	-	-	++	-	-	6.84
Large Stigma A/Giza 181 R	F_1	-	++	+	-	-	-26.85**
IR 68885 A/Giza 181 R	F_1	-	+++	-	+	-	20.28**
Giza 181 R	Male	-	++++	-	++	-	-
IR 58025 A/Giza 182 R	F_1	-	-	+++	-	-	-12.73**
G 46 A/Giza 182 R	F_1	-	+++	-	++	+	27.83**
Large Stigma A/Giza 182 R	F_1	-	+	-	-	-	-10.75*
IR 68885 A/Giza 182 R	F_1	-	+++	-	++	-	-21.22**
Giza 182 R	Male	+++	-	+++	++	-	-

- Absent + Very weak ++ Weak
+++ Intermediate ++++ Strong +++++ Very strong

** Best hybrid combinations with high standard heterosis (SH%) for grain yield over best local check inbred variety Giza 178

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الملخص العربـــــى

المشابهات الجزيئية لسلالات العقم الذكرى السيتوبلازمى الوراثى وسلالات إعادة الخصوبة وتراكيبها الهجينية بناءً على تحليل الـ RAPD والمشابهات الإنزيمية Isozymes

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نظـــام العقـــم الذكرى السيتوبلازمى الوراثى هو حجر الزاوية لتربية الهُجـــن على مستوى العالم كذلك المحافظة على التنوع الوراثى للأصول الوراثية لسلالات العقم الذكرى السيتوبلازمى لتقليل التدهور الوراثى **Vulnerability** نتيجة إنكســـار المقاومـــة للأمـــراض والحشرات عند إستخدام مصدر واحد فقط وزيادة إحتمالـــية تطوير أو تحسين تراكيب هجينية جيدة . فإستخدام سلالات عُقم ذكرى ســـيتوبلازمى من مصادر عقم مختلفة أعطى مدى واسع من التنوع إستُخدم حالياً كأصـــول وراثية محلية للأرز الهجين . وفى هذه الدراسة تم إستخدام الـــ **RAPD** للمعلـــم والمشـــابهات الإنزيمـــية **Isozymes** بغرض تحديد كشافات وراثية جزئية وبيوكيميائـــية ودراســـة التباين الوراثى بين أربع سلالات عقم ذكرى سيتوبلازمى وراثى متنوعة المصادر وثلاث أصناف مصرية معيدة للخصوبة مختارة وتستخدم على نطاق واسع فى برامج تربية الأرز الهجين ، علاوة على الهُجن الناتجة بينها ودراسة العلاقة مع قوة الهُجن .

وقـــد تـــم عزل الـــ **DNA** من النباتات للأربع مجاميع المختلفة من الأرز (كـــلا منها يمثل السلالة العقيمة ذكرياً والسلالة المعيدة للخصوبة وهجين الـــ F_1) وتم تكبيره بواسطة تكنيك الـــ **PCR** باستخدام بوادئ عشوائية مختلفة (تحليل الـــ

(RAPD) . وبعـد ذلك تم تحليل الـ DNA بواسطة التفريد الكهربى بالأجاروس جيل وتم تحديد أحجام حزم الـ DNA الموجودة والغائبة . من بين الخمس بوادئ المسـتخدمة أعطـت أربعة منهم إختلافات فى الشكل المظهرى للـ DNA . وقد أظهـر البادئ 9050309 (5 GTG GGA ATG TGC GCT 3) حـزم DNA متضـاعفة أكـثر من مختلف التراكيب الوراثية . وقد ظهرت حزمتين رئيسيتين بأحجـام أكـبر من 2600 bp < و 2100 bp تقريباً فى نباتات الأصناف المعيدة لخصـوبة جـيزة ١٧٨ أر وجـيزة ١٨١ أر ، بينما غابت نفس الحزم فى جميع السـلالات ذات العقم الذكرى السيتوبلازمى الأربع والصنف جيزة ١٨٢ أر المعيد للخصـوبة . وقد تم تحديد حزمة واحدة فقط ذات حجم 700 bp الخاصة بالبادئ 9050322 فى الأباء المعيدة للخصوبة جيزة ١٧٨ أر وجيزة ١٨٢ أر بينما غابت فى الأب جيزة ١٨٢ أر . ربما يُستدل من ذلك على وجود جينين من جينات إعادة الخصوبة R فى الصنف جيزة ١٧٨ أر وجين واحد مختلف فى كل من الصنف جيزة ١٨١ أر وجيزة ١٨٢ أر .

تحلـيل المشـابهات الإنزيمـية لإنـزيمى الإستيريز والبيروكسيديز يمكن إسـتخدامه ايضـا كعلامات وراثية بيوكيميائية للسلالات الأبوية وتوليفات هُجن الجـيل الأول . فقد أظهرت الحزم رقم ٥ ، ٦ ، ٧ للإستيريز و ١ ، ٤ ، ٥ ، ٦ ، ١٠ للبيروكسيديز إختلافات متباينة من تركيب وراثى لأخر . وقد أظهرت النتائج أيضا وجود العديد من الطرز التى تشير إلى قوة الهجين فى الأرز ـــ وتعتبر الحزم المتكاملة أكثرها أهمية .