Morphological and Molecular Genetical Studies on Restoring Ability for WA Type CMS Lines in Rice (*Oryza sativa* L.)

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ABSTRACT

Three cytoplasmic male sterile lines wild abortive (WA); IR 58025 A, IR 69625 A and IR 70368 A were tested with nine different testers for some morphological, biochemical and molecular studies. A total of 27 F_1 crosses were made and harvested separately for each cross. The results appeared a wide range of variations was recorded for yield and related traits among the parental lines with their crosses. This means that the direction of heterosis was negative for some traits such as days to heading. On the other hand, positive heterosis was recorded for the majority of remained characters. With respect to general combining ability effects, the CMS line IR 69625 A was the best combiner for grain yield / plant. Also, for straw weight, IR 58025 A and IR 70368 A recorded significant and highly significant negative differences, respectively. But for specific combining ability effects, two crosses IR 58025 A x IRGA 370- 42- 1- 1F- C-1 and IR 69625 A x Giza 175 recorded significant and positive values for No. of tillers. In the peroxidase zymogram, results showed that, sub-patterns were observed among the different tested genotypes; these have specific bands which could be used to distinguish among the three CMS lines. While in respect of the esterase zymogram, a complex of sub-patterns was observed among the different tested genotypes. Total soluble proteins differences were detected in the CMS lines IR 58025 A and IR 69625 A and their hybrids. Generally, some bands were missing in the CMS lines,

while present in most of the hybrids ,as well as, the testers. These bands might be derived from the testers. From DNA studies, a level of molecular polymorphism was detected. For example, band no. 7 was found in many testers and F_1' S in the two CMS lines; IR 58025 A and IR 70368 A, in spite of its missing in the CMS line itself. Therefore, further experiments could be applied for studying the correlation of this band with the restoring ability of CMS lines.

INTRODUCTION

Rice (*Oryza sativa*, L.) is considered as one of the most important cereal crops not only in Egypt, but also all over the world. In Egypt the yearly area cultivated annually with rice is almost more than one fifth of the total area.

Hybrid varieties developed by making use of WA CMS account for approximately 99% of the hybrid rice produced in China. These varieties have significantly outyielded conventional pure line cultivars. The WA CMS system has also been used extensively in hybrid rice breeding programs of other countries to produce hybrids in various genetic backgrounds (Virmani, 1994).

The wild abortive (WA) type of CMS source is used extensively for hybrid rice seed production, in China ,as well as, in tropical rice-growing areas.

Many scientists in China have studied the relationship between isozymes and heterosis in rice. They found that the hybrid combination whose F_1 had dominant complementary bands for the esterase isoenzyme was closely associated with expression of heterosis Kato *et al.* (1994), Li *et al.*, (1996), Banumathy *et al.* (2003) and Chakraborty *et al.* (2002). The complementary enzyme bands may be used as one of the biochemical indicators for predicting heterosis.

Molecular marker studies have also been employed to determine the map positions of the fertility restorer genes. Zhang *et al.* (1996) surveyed DNA polymorphism using randomly amplified polymorphic DNA (RAPD) analysis in a set of near-isogenic lines (NIL_S) developed by introgressing of the fertility restorer genes from IR 24 into the genetic background of Zhenshan 97 (Zhang *et al.*, 1997).

This study was undertaken to achieve the following objectives:

i. Estimation of heterosis and combining ability for morphological, yield and its components in parental lines and their F_1 hybrids.

ii. Study of the relationship between isozymes and total soluble protein and restoring ability in rice.

iii. Using RAPD markers to identify the relationship between morphological and yield characters and molecular markers.

MATERIALS AND METHODS

This study was carried out at the experimental farm and Biotechnology laboratory of the Rice Research and Training Center (RRTC), Sakha, Kafr EL-Sheikh, Egypt and laboratories of Genetics Department, Faculty of Agriculture, Kafr EL-Sheikh University, during the two growing seasons 2002 and 2003. Three cytoplasmic male sterile lines; wild abortive (WA) IR 58025 A, IR 69625 A and IR 70368 A were tested with nine testers; Giza 175, Giza 178, Giza 181, Giza 182, IRGA 370-2-1 - 1F-C-1, WAB 326 - B-B-15, IR 69312-14-2-2-2,
CT 9685-14-M-1-2-3-2P-M-1 and IET 12515.

Parents and hybrids were used to study the genetic analysis of yield and its components. These components were: plant weight, panicle length, grain yield per plant, 1000 grain weight and harvest index %. Some morphological traits such as days to flowering, plant height and number of tillers per plant were studied.

Biochemical analysis:

For isozymes detection, the method of Scandalios (1969) was used for the detection of esterase, and the method described by Tu *et al.* (1968) was used for the detection of peroxidase.

PCR based RAPD Analysis:

DNA isolation and purification was carried out using CTAB (Cetyl-tetramethyl ammonium bromide) method, (Murray and Thompson 1980).

DNA quantification:

DNA was quantified using gel quantification method, in which the samples were loaded on 0.8% agarose gel in 0.5 x TAE buffer and using diluted λ genomic DNA as standard. The intensity of individual sample was compared with a range of known amounts of λ DNA (25, 50, 75 and 100 ng). According to the sample intensity, samples were diluted, and again loaded on 0.8% gels till all of the samples finally reached around 50 ng/μl in a uniform manner.

The five 10-mer oligonucleotide random primers used in this study were as follows:

Primer	Sequence
RBL.4	$\bar{5}$- AGC TTG CCC C- $\bar{3}$
RBL.5	$\bar{5}$- GAA GGC GGT G- $\bar{3}$
RBL.6	$\bar{5}$- GGG GTA CCG T- $\bar{3}$
RBL.7	$\bar{5}$- CGT CAA GCC C- $\bar{3}$
RBL.8	$\bar{5}$- GAG GCG GCT T- $\bar{3}$

RBL, Rice Biotechnology Laboratory (RRTC)

PCR amplification condition used in this part in 20 μl PCR volume containing 1.0 μl (50ng template DNA), 0.2 μl dNTPs, (10 mM), 1.6 μl $MgCl_2$ (25 mM), 2.0 μl 10X buffer (10 mM tris, pH 8.0, 50mM KCl and 50 mM ammonium sulphate), 4.0 µl primer (15 pmole), 0.1 µl taq polymerase (10u/ µl). The volume was brought up to 20 µl by autoclaved double distilled H_2O.

Statistical Analysis:

Twenty-seven hybrid combinations were produced from three lines and nine diverse testers. The data were subjected to analysis of variance for randomized complete block design as suggested by Panse and Sukhatme (1954) and analysis of variance for line x tester crossing according to Kempthorne (1957). All the present data were subjected to arcsine transformation before analysis.

Estimation of combining ability:

Assuming there are (L) lines and (t) testers and data are obtained by plot mean, two methods of analysis according to Kempthorne (1957) and Virmani *et al.* (1997) were used.

Estimation of heterosis:

$$\text{Heterosis over the mean parent \% } (\overline{MP}) = \frac{F_1 - \overline{MP}}{\overline{MP}} \times 100$$

Where: F_1 = mean value of the first generation and $\overline{MP}$ = mid. parent value.

RESULTS AND DISCUSSION

The development of hybrid rice technology and adoptions of hybrid rice cultivars to Egyptian environments offer one approach to the problem of matching food supply to expected demand.

Mean performance:

A wide range of differences among the parental lines and their crosses are shown in Tables (1 and 2), and for each trait there were significant differences. The data in Table (1) showed that, the mean values of the testers

Table (1): Mean values for some studied characters for parents.

Testers	Days to heading (day)	Plant height (cm)	No. of tillers	Panicle length (cm)	Grain yield/plant (g)	Straw weight/plant (g)	Harvest index (%)	1000- grain weight (g)
Giza 175	95.00	95.00	19.00	22.02	23.72	47.40	41.36	17.67
Giza 178	100.00	96.76	16.33	20.27	15.08	33.05	35.96	19.87
Giza 181	112.00	97.67	20.00	24.80	38.04	59.73	40.80	23.83
Giza 182	92.00	70.00	14.67	22.40	62.09	67.70	59.21	25.17
IRGA 370-42-1-1F-C-1	100.00	97.13	20.33	19.53	25.89	65.15	27.12	22.83
WAB 326-B-B-15	94.00	109.87	10.67	20.60	28.12	54.84	35.46	24.13
CT9685-14-M-1-2-3-2P-M-1	102.00	103.47	19.00	22.93	41.46	59.63	42.08	27.00
IET 12515	104.00	103.07	18.33	20.07	21.36	51.95	32.96	22.23
IR 69312-14-2-2-2	99.00	92.47	10.33	21.93	19.40	24.72	66.90	26.90
Mean	99.78	96.15	16.52	21.62	30.57	51.57	42.43	23.29
Lines	**Days to heading (day)**	**Plant height (cm)**	**No. of tillers**	**Panicle length (cm)**	**Grain yield/plant (g)**	**Straw weight/plant (g)**	**Harvest index (%)**	**1000- grain weight (g)**
IR 58025 A	107	87.27	24.67	21.67	36.21	64.34	36.01	20.50
IR 69625 A	100	91.33	26.00	22.33	46.30	72.87	38.84	24.20
IR 70368 A	101	84.27	18.33	23.27	41.51	54.49	43.24	25.40
Mean	102.67	87.62	23.00	22.42	41.34	63.90	39.36	23.37
L.S.D at 5%	3.14	15.62	3.16	1.34	6.20	6.94	8.08	1.92
L.S.D at 1%	4.18	20.77	4.20	178	9.23	9.23	10.75	2.55

Table (2): Mean values for some studied characters for the crosses.

No	Crosses	Days to heading (day)	Plant height (cm)	No. of tillers	Panicle length (cm)	Grain yield / plant (g)	Straw weight/plant (g)	Harvest index	1000- grain weight (g)
1	IR58025A x Giza 175	94.03	102.47	26.00	23.60	44.07	61.00	38.99	19.63
2	IR58025A x Giza 178	95.33	107.93	18.67	21.13	52.59	70.61	57.97	19.43
3	IR58025A x Giza 181	107.67	108.93	18.00	23.73	59.09	103.46	38.33	18.00
4	IR58025A x Giza 182	95.67	95.40	21.67	22.73	48.03	57.93	60.94	23.87
5	IR58025A x IRGA 370-42-1-1F-C-1	97.33	91.27	37.67	25.00	33.47	58.40	39.24	25.27
6	IR58025A x WAB 326-B-B-1	90.33	122.80	33.00	25.80	26.26	84.50	23.65	29.23
7	IR58025A x CT9685-14-M-1-2-3-2P-M-1	101.00	103.07	19.33	22.33	22.35	53.95	37.47	18.10
8	IR58025A x IET 12515	101.03	96.33	21.33	23.93	27.70	54.15	36.63	25.23
9	IR58025A x IR 69312-14-2-2-2	101.00	96.07	30.00	25.00	13.61	121.67	10.10	8.00
10	IR 69625 A x Giza 175	94.67	101.33	36.33	22.47	89.59	126.95	39.53	23.00
11	IR 69625 A x Giza 178	100.33	108.53	25.67	24.80	47.41	66.03	51.11	24.27
12	IR 69625 A x Giza 181	94.33	96.07	22.33	21.60	42.02	48.26	50.17	24.93
13	IR 69625 A x Giza 182	94.67	100.40	28.00	22.40	57.96	63.12	55.13	27.37
14	IR 69625 A x IRGA 370-42-1-1F-C-1	94.33	97.60	25.00	21.87	57.38	67.12	42.36	22.73
15	IR 69625 A x WAB 326-B-B-15	93.03	133.73	19.33	22.00	24.23	126.03	15.79	27.10
16	IR 69625 A x CT9685-14-M-1-2-3-2P-M-1	95.33	100.33	20.33	23.07	44.97	83.56	30.18	25.10
17	IR 69625 A x IET 12515	99.33	105.80	18.67	21.73	20.34	149.22	12.28	20.13
18	IR 69625 A x IR 69312-14-2-2-2	99.33	99.13	24.33	22.33	12.73	129.16	8.80	11.77
19	IR 70368 A x Giza 175	94.03	98.20	16.67	23.53	49.70	53.39	54.50	23.13
20	IR 70368 A x Giza 178	101.33	104.73	24.67	23.47	50.26	56.84	50.06	25.10
21	IR 70368 A x Giza 181	103.03	96.27	17.67	22.40	45.56	50.44	49.60	26.22
22	IR 70368 A x Giza 182	91.33	100.13	15.67	22.80	41.72	55.08	44.47	26.93
23	IR 70368 A x IRGA 370-42-1-1F-C-1	93.67	98.20	18.33	23.80	25.93	53.63	48.68	25.63
24	IR 70368 A x WAB 326-B-B-15	89.03	130.60	22.00	22.73	45.54	74.82	41.79	28.47
25	IR 70368 A x CT9685-14-M-1-2-3-2P-M-1	97.67	103.80	19.00	22.00	44.34	65.70	50.29	26.53
26	IR 70368 A x IET 12515	102.03	88.73	24.00	23.00	11.55	79.00	13.00	10.50
27	IR 70368 A x IR 69312-14-2-2-2	101.67	93.00	24.67	23.67	12.76	122.83	10.14	8.03
	Mean.	97.13	102.99	23.27	23.11	38.93	79.15	37.45	21.99

were (99.78, 96.15, 30.57, 42.43 and 23.29) for days to heading, plant height, grain yield per plant, harvest index and 1000-grain weight. But the mean performances of their crosses for these traits were 97.13, 102.99, 38.93, 37.45 and 21.99, respectively (as shown in Table 2).

For the CMS lines, the results indicated that the IR 69625 A gave the desirable values for days to heading (100), No. of tillers /plant (26.00) and grain yield/plant (46.30). While, the IR 70368 A gave the desirable values (84.27, 23.27, 54.49, 43.24 and 25.40) for plant height, panicle length, straw weight, harvest index and 1000-grain weight respectively.

Analysis of variance:

Results in Table (3) showed that analysis of variance revealed highly significant differences among the 39 genotypes (27 hybrids, 9 testers and 3 CMS lines) tested for all the studied parameters. The parental lines and the hybrids showed highly significant differences for some traits. Parents v.s crosses mean squares indicated that the average heterosis were significant in all crosses for all studied traits.

Heterosis :

Evaluation based on the standard heterosis revealed that15 hybrids recorded significantly negative standard heterosis for early heading date. These findings indicated that heterosis effects can be used to get earliness in rice hybrids. The highest estimates of the 15 hybrids were recorded for the crosses IR 70368 A x WAB 326-B-B15, IR 58025 A x WAB 326-B-B-15 and IR 70368 x Giza 182 which recorded -11.00%, -10.00% and -9.00%, respectively (Table 4). While 21 cross of the 27 crosses recorded significantly positive standard heterosis for grain yield/plant (g). The highest evaluates were IR 69625A x Giza 175, IR 58025A x Giza 181 and IR 69625A x Giza 182 which recorded 494.10, 291.84 and 284.42, respectively (Table 4 cont).

Table (3): Mean square values of lines, testers and their crosses for some studied characters.

Source of variance	d . f	Days to heading (day)	Plant height (cm)	No. of tillers	Panicle length (cm)	Grain yield / plant (g)	Straw weight/plant (g)	Harvest index	1000-grain weight (g)
Replication	2	0.718^{ns}	75.820^{ns}	2.872^{ns}	3.176^{*}	4.227^{ns}	44.104^{ns}	9.427^{ns}	0.923^{ns}
Genotype	38	72.541^{**}	382.926^{**}	104.305^{**}	5.768^{**}	853.652^{**}	2439.752^{**}	661.287^{**}	83.988^{**}
Parents	11	10.212^{**}	35.777^{ns}	7.465^{*}	0.761^{ns}	60.728^{**}	66.040^{**}	40.802^{ns}	2.697^{*}
Crosses	26	6.613^{*}	38.460^{ns}	10.974^{**}	0.439^{ns}	109.509^{**}	304.399^{**}	88.259^{**}	12.311^{**}
Parents V.S Crosses	1	2472.280^{**}	13157.690^{**}	3596.154^{**}	199.406^{**}	28923.336^{**}	84069.772^{**}	22385.363^{**}	2841.783^{**}
Lines (GCA)	2	3.346^{ns}	8.127^{ns}	20.200^{**}	1.338^{ns}	59.402^{*}	628.397^{**}	$31.36.^{ns}$	3.766^{ns}
Testers (GCA)	8	14.077^{**}	108.032^{ns}	8.788^{*}	0.166^{ns}	238.410^{**}	477.989^{**}	227.887^{**}	29.911^{**}
Lines x Testers (SCA)	16	3.290^{ns}	7.466^{ns}	10.914^{**}	0.463^{ns}	51.335^{**}	177.104^{**}	25.557^{ns}	4.579^{**}
Error	76	3.727	91.465	3.731	0.670	14.447	18.011	24.424	1.388

* , ** : Significant at 5% and 1% , respectively.
ns : Not significant .

Table (4): Estimates of standard heterosis (S.H), better parent ($\overline{BP}$) and mid – parent ($\overline{MP}$) for some studied characters.

No	Crosses	Days to heading			Plant height (cm)			No. of tillers			Panicle length		
		S.H%	BP %	MP %	S.H%	BP %	MP %	S.H%	BP %	MP %	S.H%	BP %	MP %
1	IR58025A x Giza 175	-6.00**	-1.05ns	-6.93**	6.00ns	17.42ns	12.44ns	59.22**	5.93ns	19.07**	16.43**	6.93*	7.91**
2	IR58025A x Giza 178	-5.00**	-5.00**	-8.21**	11.66ns	23.67*	17.35ns	14.27ns	-24.32**	-8.93ns	4.29ns	-2.49ns	0.76ns
3	IR58025A x Giza 181	8.00**	0.93ns	-1.37ns	12.69ns	24.82*	17.80*	10.23ns	27.04**	-19.41**	17.12**	-4.31ns	2.13ns
4	IR58025A x Giza 182	-4.00**	4.35**	-3.52*	-1.31ns	36.29**	21.32*	32.64**	-12.16ns	10.17ns	12.19**	1.47ns	3.15ns
5	IR58025A x IRGA 370-42-1-1F-C-1	-3.00*	-3.00**	-6.28**	-5.39ns	4.58ns	-1.01ns	130.62**	52.70**	67.42**	23.33**	15.37**	21.36**
6	IR58025A x WAB 326-B-B-15	-10.00**	-4.26**	-10.45**	27.03**	40.71**	24.58**	102.08**	33.77**	86.76**	27.28**	19.06**	22.07**
7	IR58025A x CT9685-14-M-1-2-3-2P-M-1	1.00ns	-0.98ns	-3.35*	6.62ns	18.10*	8.07ns	18.37ns	-21.65**	-11.47ns	15.15**	1.74ns	4.62ns
8	IR58025A x IET 12515	1.00ns	-2.88*	-4.27**	-0.34ns	10.38ns	1.72ns	30.62**	-13.54*	0.79ns	18.11**	10.43**	14.66**
9	IR58025A x IR 69312-14-2-2-2	1.00ns	2.02ns	-1.94ns	-0.62ns	10.08ns	6.90ns	83.71**	21.61**	71.43**	23.33**	14.00**	14.68**
10	IR 69625 A x Giza 175	-5.00**	0.00ns	-2.56ns	4.83ns	10.95ns	8.76ns	122.47**	39.73**	61.47**	10.85**	0.63ns	1.22ns
11	IR 69625 A x Giza 178	0.00ns	0.00ns	0.00ns	12.28ns	13.83*	15.46*	57.13**	-1.27ns	21.29**	22.35**	11.06**	16.43**
12	IR 69625 A x Giza 181	-6.00**	-6.00**	-11.32**	-0.62ns	5.19ns	1.66ns	36.47**	-1.41*	-2.91ns	6.65*	-12.90**	-8.34**
13	IR 69625 A x Giza 182	-5.00**	3.26*	-1.04ns	3.86ns	9.93ns	24.47**	71.46**	7.69ns	37.69**	10.51**	0.00ns	0.16ns
14	IR 69625 A x IRGA 370-42-1-1F-C-1	-6.00**	-6.00**	-6.00**	0.96ns	6.87ns	3.58ns	53.09**	-3.85ns	7.92ns	7.89*	-2.06ns	4.49ns
15	IR 69625 A x WAB 326-B-B-15	-7.00**	-1.06ns	-4.12*	38.35**	46.42**	32.93**	18.37ns	-25.65**	5.43ns	8.53*	-1.48ns	2.49ns
16	IR 69625 A x CT9685-14-M-1-2-3-2P-M-1	-5.00**	-5.00**	-5.94**	3.30ns	9.85ns	3.01ns	24.49*	-21.81**	-9.64ns	13.81**	0.61ns	1.94ns
17	IR 69625 A x IET 12515	-1.00ns	-1.00ns	-2.94ns	9.44ns	15.84ns	8.85ns	14.27ns	-28.19**	-15.77*	7.25*	-2.69ns	2.50ns
18	IR 69625 A x IR 69312-14-2-2-2	-1.00ns	0.00ns	-0.50ns	2.56ns	8.54ns	7.87ns	48.99**	-6.42ns	33.94**	10.21**	0.00ns	0.90ns
19	IR 70368 A x Giza 175	-6.00**	-1.05ns	-4.08*	1.58ns	16.53ns	9.56ns	2.02ns	-12.26ns	-10.69ns	16.13*	1.12ns	3.79ns
20	IR 70368 A x Giza 178	1.00ns	1.00ns	0.50ns	8.35ns	24.28*	15.76*	51.01**	34.59**	42.35**	15.79**	0.86ns	7.81**
21	IR 70368 A x Giza 181	3.00*	1.98ns	-3.29*	-0.41ns	14.24ns	5.83ns	8.14ns	-11.65ns	-7.80ns	10.51**	-9.68**	-6.80**
22	IR 70368 A x Giza 182	-9.00**	-1.09ns	-5.70**	3.59ns	43.04**	29.81**	-4.10ns	-14.51ns	-5.03ns	12.48**	-2.02ns	-0.15ns
23	IR 70368 A x IRGA 370-42-1-1F-C-1	-6.00**	-6.00**	-6.47**	1.58ns	16.53ns	8.27ns	12.25ns	-9.84ns	-5.17ns	17.41**	2.28ns	11.21**
24	IR 70368 A x WAB 326-B-B-15	-11.00**	-5.32**	-8.72**	35.10**	54.98**	34.54**	34.72**	20.02*	51.72**	12.19**	-2.32ns	3.62ns
25	IR 70368 A x CT9685-14-M-1-2-3-2P-M-1	-2.00ns	-2.97*	-3.45*	7.38ns	23.18*	10.38ns	16.35ns	0.00ns	1.79ns	8.53*	-5.46ns	-4.76ns
26	IR 70368 A x IET 12515	2.00ns	0.99ns	-0.49ns	-8.20ns	5.29ns	-5.27ns	46.97**	30.93**	30.93**	13.47**	-1.16ns	6.14*
27	IR 70368 A x IR 69312-14-2-2-2	2.00ns	3.03*	2.00ns	-3.80ns	10.36ns	5.24ns	51.01**	34.59**	72.16**	16.77**	1.72ns	4.73ns
	L.S.D at 5%	3.00	3.14	2.74	15.55	15.02	13.52	3.14	3.16	2.74	1.33	1.34	1.16
	L.S.D at 1%	4.00	4.18	3.64	20.68	20.27	17.98	4.17	4.28	3.64	1.77	1.78	1.54

*, ** : Significant at 5% and 1%, respectively.
ns : Not significant.

Table (4): Cont. Estimates of standard heterosis (S.H), better parent ($\overline{BP}$) and Mid – parent ($\overline{MP}$) for some studied characters.

No	Crosses	Grain yield /plant(g)			Straw weight/plant (g)			Harvest index			1000 grain weight (g)		
		S.H%	BP %	MP %	S.H%	BP %	MP %	S.H%	BP %	MP %	S.H%	[illegible]	[illegible]
1	IR58025A x Giza 175	192.24**	21.71^{ns}	47.05**	84.57**	28.69**	9.18^{ns}	8.43^{ns}	-5.73^{ns}	0.77^{ns}	-1.16^{ns}	-4.24^{ns}	2.83^{ns}
2	IR58025A x Giza 178	248.74**	45.24**	15.03**	113.65**	113.65**	44.99**	61.21**	60.98**	61.07**	-2.16^{ns}	-5.22^{ns}	-3.76^{ns}
3	IR58025A x Giza 181	291.84**	55.34**	59.14**	213.07**	73.21**	66.76**	6.59^{ns}	-6.05^{ns}	-0.21^{ns}	-9.41^{ns}	-24.46**	-18.81**
4	IR58025A x Giza 182	218.50**	-22.64**	-2.28^{ns}	75.31**	-9.96^{ns}	-12.25**	69.47**	2.92^{ns}	28.00**	20.13**	-5.16^{ns}	4.51^{ns}
5	IR58025A x IRGA 370-42-1-1F-C-1	121.95**	-7.57^{ns}	7.79^{ns}	76.70**	-9.23^{ns}	-9.81*	9.12^{ns}	8.97^{ns}	24.30^{ns}	27.18**	10.69*	16.61**
6	IR58025A x WAB 326-B-B-15	74.20**	-27.48**	-18.37*	155.67**	54.08**	41.80**	-34.23**	-34.32**	-33.83**	47.16**	21.14**	30.96**
7	IR58025A x CT9685-14-M-1-2-3-2P-M-1	48.21^{ns}	46.09**	-42.46**	63.24**	-9.53^{ns}	-12.97**	4.20^{ns}	-10.96^{ns}	-4.05^{ns}	-8.91^{ns}	-32.96**	-24.11**
8	IR58025A x IET 12515	83.69**	-23.50**	-3..79^{ns}	63.84**	4.23^{ns}	-6.88^{ns}	1.86^{ns}	1.72^{ns}	6.20^{ns}	26.98**	13.50**	18.01**
9	IR58025A x IR 69312-14-2-2-2	-9.75^{ns}	-62.41**	-51.06**	268.41**	392.56**	173.43**	-71.91**	-84.90**	-80.37**	-59.74**	-70.26**	-66.24**
10	IR 69625 A x Giza 175	494.10**	93.50**	155.90**	284.11**	167.83**	111.09**	9.39^{ns}	-4.42^{ns}	-1.42^{ns}	15.75**	-4.96^{ns}	9.84**
11	IR 69625 A x Giza 178	214.39**	2.40^{ns}	54.48**	99.79**	99.79**	24.68**	42.13**	31.59**	36.66**	22.14**	0.29^{ns}	10.12**
12	IR 69625 A x Giza 181	178.65**	-9.24^{ns}	-0.36^{ns}	46.05**	-19.20**	-27.21**	39.52**	22.97*	25.99**	25.52**	3.02^{ns}	3.79^{ns}
13	IR 69625 A x Giza 182	284.42**	-6.65^{ns}	6.94^{ns}	90.98**	-6.77^{ns}	-10.20**	53.31**	-6.89^{ns}	12.44^{ns}	37.75**	8.74*	10.85**
14	IR 69625 A x IRGA 370-42-1-1F-C-1	280.50**	23.93**	58.95**	103.09**	3.02^{ns}	-2.74^{ns}	17.80^{ns}	9.66^{ns}	28.44**	14.44**	-6.07^{ns}	-3.36^{ns}
15	IR 69625 A x WAB 326-B-B-15	60.68*	-47.67**	-34.88**	281.36**	129.81**	97.35**	-56.09**	-59.35**	-57.50**	36.39**	11.98**	12.12**
16	IR 69625 A x CT9685-14-M-1-2-3-2P-M-1	198.28**	-2.87^{ns}	2.48^{ns}	152.83**	40.13**	26.13**	-16.07^{ns}	-28.28**	-25.41**	26.32**	-7.04^{ns}	-1.95^{ns}
17	IR 69625 A x IET 12515	34.95^{ns}	-56.07**	-39.88**	351.50**	187.24**	139.10**	-65.85**	-68.38**	-65.79**	1.36^{ns}	-16.82**	-13.31**
18	IR 69625 A x IR 69312-14-2-2-2	-15.58^{ns}	-72.51**	-61.25**	290.80**	422.49**	164.67**	-75.53**	-86.85**	-83.36**	-40.76**	-56.24**	-53.93**
19	IR 70368 A x Giza 175	229.58**	19.73*	52.36**	61.57**	12.64^{ns}	4.79^{ns}	51.56**	26.04**	28.84**	16.46**	-8.94*	7.38^{ns}
20	IR 70368 A x Giza 178	233.29**	21.08**	77.60**	71.98**	71.98**	29.86**	39.21**	15.77^{ns}	26.41**	26.32**	-1.18^{ns}	10.87**
21	IR 70368 A x Giza 181	202.19**	9.76^{ns}	14.53*	52.65**	-7.43^{ns}	-11.68*	37.03**	14.72^{ns}	18.04*	32.06**	3.27^{ns}	6.54^{ns}
22	IR 70368 A x Giza 182	176.72**	-32.81**	-19.46**	66.69**	1.08^{ns}	-9.35*	23.67*	-24.89**	-13.20^{ns}	35.58**	6.02^{ns}	6.48^{ns}
23	IR 70368 A x IRGA 370-42-1-1F-C-1	72.02*	-37.53**	-23.06**	62.27**	-1.58^{ns}	-10.35*	35.37**	12.58^{ns}	38.37**	29.04**	0.91^{ns}	6.26^{ns}
24	IR 70368 A x WAB 326-B-B-15	202.06**	9.71^{ns}	30.79*	126.41**	37.31**	36.86**	16.21^{ns}	-3.35^{ns}	6.20^{ns}	43.28**	12.09**	14.49**
25	IR 70368 A x CT9685-14-M-1-2-3-2P-M-1	194.03**	6.82^{ns}	6.87^{ns}	98.82**	20.57**	15.14**	39.85**	16.30^{ns}	17.89*	33.57**	-1.74^{ns}	1.26^{ns}
26	IR 70368 A x IET 12515	-23.41^{ns}	-72.18**	-63.26**	139.03**	52.07**	48.44**	-63.85**	-69.94**	-65.88**	-47.16**	-58.66**	-55.92**
27	IR 70368 A x IR 69312-14-2-2-2	-15.32^{ns}	-69.26**	-58.11**	271.65**	396.89**	210.10**	-71.80**	-84.94**	-81.59**	-59.54**	-70.15**	-69.29**
	L.S.D at 5%	6.18	3.14	5.38	6.90	6.94	6.50	8.14	9.08	[illegible]	[illegible]	[illegible]	[illegible]
	L.S.D at 1%	8.20	4.18	7.16	9.15	9.23	7.98	10.86	10.75	3.28	[illegible]	[illegible]	[illegible]

*, ** : Significant at 5% and 1%, respectively.
ns : Not significant.

Patel *et al.* (1994) reported that, the heterosis effects were high for grain yield / plant and similar reports have been made by Yolanda and Das (1996) for days to flowering. The results obtained by Panwar *et al.* (1998), Ramesha *et al.* (1998), Vishwakarma *et al.* (1999), Jin *et al.* (2001), Bhave *et al.* (2002), Singh *et al.* (2002) and Banumathy *et al.* (2003) were in agreement for the other traits with those obtained in this investigation.

General combining ability effects (GCA):

Among three lines tested (Table 5 A), IR 69625 A was the best combiner for grain yield / plant. Also IR 58025 A and IR 70368 A recorded significant and highly significant negative differences, respectively for straw weight.

The results in (Table 5 B) suggested that, the variety WAB 326-B-B-15 was the best general combiner for days to heading and 1000-grain weight, Giza 175 was a good combiner for grain yield / plant, Giza 182 was a good combiner for straw weight, grain yield / plant, harvest index and 1000-grain weight and finally Giza 178 was a good combiner for grain yield / plant, straw weight and harvest index.

Specific combining ability effects (SCA):

The results summarized in (Table 6) suggested that the IR 58025 A x WAB 326-B-B-15, IR 69625 A x Giza 181 and IR 70368 A x WAB 326-B-B-15 were good combiners for days to heading based on the standard heterosis and also the selection of WAB 326-B-B-15 as a tester was good donor to give significant and negative heterosis. Three crosses exhibited significant and highly significant and positive standard heterosis over the standard parent, the

Table (5 A): General combining ability (GCA) effects of lines for some studied characters.

Lines	Days to heading (day)	Plant height (cm)	No. of tillers	Panicle length (cm)	Grain yield/plant (g)	Straw weight/plant (g)	Harvest index	1000-grain weight (g)
IR 58025 A	0.34 ^{ns}	-0.10 ^{ns}	0.60 ^{ns}	0.24 ^{ns}	-0.86 ^{ns}	-1.72 *	0.24 ^{ns}	-0.41 ^{ns}
IR 69625 A	-0.36 ^{ns}	0.59 ^{ns}	0.39 ^{ns}	-0.21 ^{ns}	1.71 *	5.45 **	-1.17 ^{ns}	0.31 ^{ns}
IR 70368 A	0.01 ^{ns}	-0.49 ^{ns}	-0.99 **	-0.02 ^{ns}	-0.86 ^{ns}	-3.72 **	0.95 ^{ns}	0.10 ^{ns}
L.S.D at 5%	0..74	3.68	0.74	0.32	1.46	1.64	1.90	0.46
L.S.D at 1%	0.98	4.89	0.98	0.43	1.94	2.18	2.53	0.61

Table (5 B): General combining ability (GCA) effects of testers for some studied characters.

Testers	Days to heading (day)	Plant height (cm)	No. of tillers	Panicle length (cm)	Grain yield/plant (g)	Straw weight/plant (g)	Harvest index	1000-grain weight (g)
Giza 175	-0.92 ^{ns}	-0.77 ^{ns}	1.02 ^{ns}	0.03 ^{ns}	7.39 **	0.44 ^{ns}	2.30 ^{ns}	-0.02 ^{ns}
Giza 178	0.53 ^{ns}	1.36 ^{ns}	-0.09 ^{ns}	0.01 ^{ns}	3.72 **	-4.88 **	5.20 **	0.31 ^{ns}
Giza 181	1.53 *	-0.86 ^{ns}	-1.32 *	-0.17 ^{ns}	3.32 *	-3.92 **	2.86 ^{ns}	0.35 ^{ns}
Giza 182	-1.03 ^{ns}	-1.45 ^{ns}	-0.50 ^{ns}	-0.15 ^{ns}	3.43 **	-6.81 **	5.36 **	1.36 **
IRGA 370-42-1-1F-C-1	-0.69 ^{ns}	-2.43 ^{ns}	1.24 ^{ns}	0.15 ^{ns}	0 ^{ns}	-6.47 **	2.00 ^{ns}	0.85 *
WAB 326-B-B-15	-2.14 **	8.68 **	0.50 ^{ns}	0.14 ^{ns}	-2.31 ^{ns}	5.33 **	-3.42 *	2.09 **
CT 9658-14-M-1-2-3-2P-M-1	0.31 ^{ns}	-0.20 ^{ns}	-1.24 ^{ns}	-0.10 ^{ns}	-0.57 ^{ns}	-3.80 **	0.62 ^{ns}	0.42 ^{ns}
IET 12515	1.20 ^{ns}	-2.01 ^{ns}	-0.65 ^{ns}	-0.07 ^{ns}	-6.36 **	4.99 **	-5.60 **	-1.12 **
IR 69312-14-2-2-2	1.20 ^{ns}	-2.31 ^{ns}	1.02 ^{ns}	0.19 ^{ns}	-8.64 **	15.15 **	-9.25 **	-4.24 **
L.S.D at 5%	1.28	6.38	1.28	0.54	2.54	2.82	3.30	0.78
L.S.D at 1%	1.70	8.49	1.70	0.72	3.38	3.75	4.39	1.04

*, ** : Significant at 5% and 1% , respectively.
ns : Not significant .

Table (6): Specific combining ability (SCA) of hybrids for some studied characters.

No	Cross	Days to heading (day)	Plant height (cm)	No. of tillers	Panicle length (cm)	Grain yield /plant (g)	Straw weight/plant (g)	Harvest index %	1000-grain weight (g)
1	IR58025A x Giza 175	-0.447^{ns}	0.697^{ns}	-0.713^{ns}	-0.103^{ns}	-4.820*	-4.767^{ns}	-2.023^{ns}	-0.357^{ns}
2	IR58025A x Giza 178	-1.563^{ns}	0.387^{ns}	-2.047^{ns}	-0.907^{ns}	1.690^{ns}	3.757^{ns}	1.403^{ns}	-0.753^{ns}
3	IR58025A x Giza 181	1.770^{ns}	2.940^{ns}	1.040^{ns}	0.140^{ns}	4.257^{ns}	13.747**	-2.803^{ns}	-1.270^{ns}
4	IR58025A x Giza 182	0.330^{ns}	-0.980^{ns}	-0.637^{ns}	-0.213^{ns}	0.460^{ns}	1.460^{ns}	2.233^{ns}	-0.323^{ns}
5	IR58025A x IRGA 370-42-1-1F-C-1	0.233^{ns}	-1.377^{ns}	2.957*	0.243^{ns}	-0.963^{ns}	1.277^{ns}	-1.640^{ns}	0.653^{ns}
6	IR58025A x WAB 326-B-B-1	-0.560^{ns}	-1.977^{ns}	2.140^{ns}	0.520^{ns}	-1.057^{ns}	-1.823^{ns}	-1.417^{ns}	0.733^{ns}
7	IR58025A x CT9685-14-M-1-2-3-2P-M-1	0.657^{ns}	0.327^{ns}	-0.677^{ns}	-0.063^{ns}	-4.100^{ns}	-2.877^{ns}	-0.850^{ns}	-1.307^{ns}
8	IR58025A x IET 12515	-0.233^{ns}	-0.110^{ns}	-0.600^{ns}	0.107^{ns}	3.473^{ns}	-11.600**	5.090^{ns}	2.610**
9	IR58025A x IR 69312-14-2-2-2	-0.233^{ns}	0.103^{ns}	0.620^{ns}	0.203^{ns}	1.057^{ns}	0.777^{ns}	-0.103^{ns}	-0.013^{ns}
10	IR 69625 A x Giza 175	0.587^{ns}	-0.373^{ns}	2.940*	-0.030^{ns}	7.783^{ns}	10.047**	-0.433^{ns}	0.047^{ns}
11	IR 69625 A x Giza 178	0.803^{ns}	-0.103^{ns}	0.497^{ns}	0.767^{ns}	-2.607^{ns}	-4.940*	-0.527^{ns}	0.140^{ns}
12	IR 69625 A x Giza 181	-2.197^{ns}	-2.037^{ns}	0.613^{ns}	-0.120^{ns}	-3.873^{ns}	-11.823**	2.553^{ns}	0.320^{ns}
13	IR 69625 A x Giza 182	0.797^{ns}	-0.003^{ns}	1.683^{ns}	0.127^{ns}	1.200^{ns}	-3.980^{ns}	1.707^{ns}	0.123^{ns}
14	IR 69625 A x IRGA 370-42-1-1F-C-1	0.023^{ns}	0.043^{ns}	-1.057^{ns}	-0.350^{ns}	4.437*	-3.987^{ns}	0.810^{ns}	-0.913^{ns}
15	IR 69625 A x WAB 326-B-B-15	1.140^{ns}	0.977^{ns}	-2.207^{ns}	-0.297^{ns}	-4.303^{ns}	4.850^{ns}	-2.627^{ns}	-0.697^{ns}
16	IR 69625 A x CT9685-14-M-1-2-3-2P-M-1	-0.643^{ns}	-1.277^{ns}	-0.133^{ns}	0.300^{ns}	0.870^{ns}	-0.177^{ns}	-1.870^{ns}	0.307^{ns}
17	IR 69625 A x IET 12515	-0.200^{ns}	2.357^{ns}	-1.277*	-0.177^{ns}	-1.550^{ns}	12.920**	-1.617^{ns}	0.190^{ns}
18	IR 69625 A x IR 69312-14-2-2-2	-0.200^{ns}	0.433^{ns}	-1.060^{ns}	-0.247^{ns}	-1.807^{ns}	-3.927^{ns}	0.873^{ns}	0.520^{ns}
19	IR 70368 A x Giza 175	-0.117^{ns}	-0.337^{ns}	-2.233*	0.133^{ns}	-2.943^{ns}	-5.303*	2.437^{ns}	0.300^{ns}
20	IR 70368 A x Giza 178	0.767^{ns}	-0.290^{ns}	1.543^{ns}	0.133^{ns}	0.913^{ns}	1.167^{ns}	-1.943^{ns}	0.627^{ns}
21	IR 70368 A x Giza 181	0.433^{ns}	-0.890^{ns}	0.440^{ns}	-0.043^{ns}	-0.253^{ns}	-1.927*	0.243^{ns}	0.963^{ns}
22	IR 70368 A x Giza 182	-0.907^{ns}	0.987^{ns}	-1.047^{ns}	0.070^{ns}	-1.643^{ns}	2.510^{ns}	-3.967^{ns}	0.187^{ns}
23	IR 70368 A x IRGA 370-42-1-1F-C-1	-0.347^{ns}	1.323^{ns}	-1.900^{ns}	0.103^{ns}	-3.477^{ns}	1.687^{ns}	0.797^{ns}	0.263^{ns}
24	IR 70368 A x WAB 326-B-B-15	-0.563^{ns}	1.013^{ns}	0.063^{ns}	-0.243^{ns}	5.370*	-3.050^{ns}	3.920^{ns}	-0.030^{ns}
25	IR 70368 A x CT9685-14-M-1-2-3-2P-M-1	-0.013^{ns}	0.960^{ns}	0.803^{ns}	-0.247^{ns}	3.230^{ns}	3.040^{ns}	2.713^{ns}	0.993^{ns}
26	IR 70368 A x IET 12515	0.430^{ns}	-2.253^{ns}	1.880^{ns}	0.057^{ns}	-1.910^{ns}	-1.317^{ns}	-3.497^{ns}	-2.810**
27	IR 70368 A x IR 69312-14-2-2-2	0.430^{ns}	-0.530^{ns}	0.423^{ns}	0.020^{ns}	0.773^{ns}	3.133^{ns}	-0.800^{ns}	-0.513^{ns}
	L.S.D at 5%	2.22	11.043	2.261	0.945	4.388	4.900	5.707	1.361
	L.S.D at 1%	2.93	14.687	2.967	1.257	[illegible]	6.518	7.589	1.809

*, ** : Significant at 5% and 1%, respectively.
ns : Not significant.

crosses were IR 58025 A x Giza 178, IR 69625 A x Giza 182 and IR 70368 A x Giza 178 in harvest index trait.

Estimates of SCA effects for the 27 rice crosses are shown in Table (6) when considering SCA effects for selection of superior hybrids, the estimate of SCA effects for number of tillers indicated that, two crosses IR 58025 A x IRGA 370- 42- 1- 1F- C-1 and IR 69625 A x Giza 175 recorded significant and positive values ranging between 2.940 and 2.957. For grain yield / plant the crosses IR 69625 A x Giza 175 (7.783), IR 70368 A x WAB 326- B- B-15 (5.370) and IR 69625 A x IRGA 370- 42- 1- 1F- C-1 (4.437) recorded significant and highly significant positive values.

From these Data, the tester Giza 182 , Giza175 and WAB 326-B-B-15 were good combiners and could be used to produce F_1 hybrid seeds when crossed with IR 69625 A which was good stable under the Egyptian conditions.

Biochemical and Molecular Analysis:

1. Isozyme Analysis:

In the peroxidase zymogram, results showed that sub-patterns were observed among the different tested genotypes, these sub-patterns have specific bands which could be used to distinguish among the three CMS lines. Where as the IR 58025 A has the band No. 7, this band was absent in their crosses except the genotype IR 58025 A x IR 69312-14-2-2-2. On the other hand, bands No. 4, 10 and 11 were absent in the IR 58025 A (Figure 1 A). Bands No. 4, 7 and 10 were also absent in the IR 69625 A (Figure 1 B), in the IR 70368 A bands No. 10 and 11 were absent (Figure 1 C). While for the testers, all the testers were differed in the number (ranged from seven to ten) and activity of the bands (Figure 2).

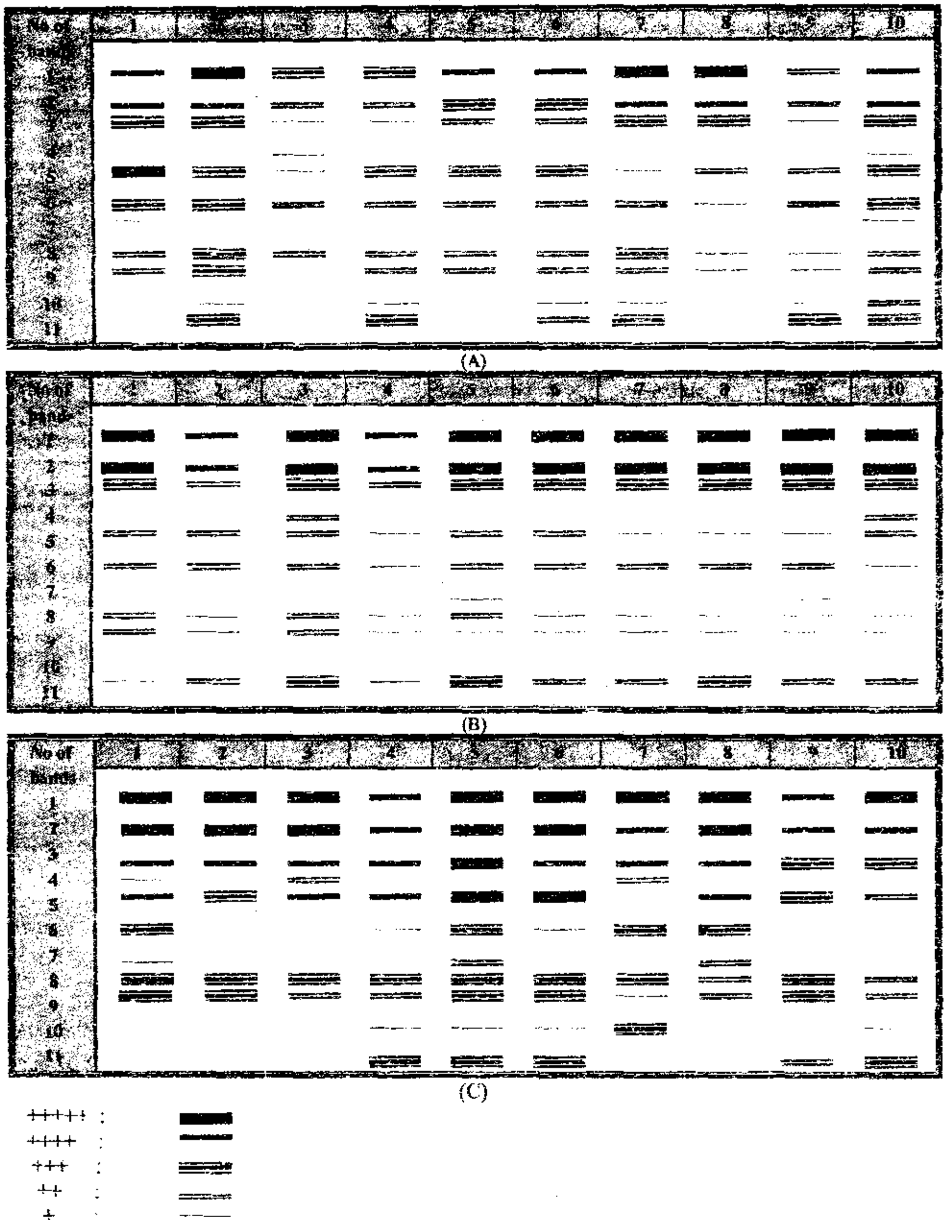


Fig. (1) Isozyme diagrams:
(A) of peroxidase for IR 58025 A (lane 1) and their nine F_1 hybrids (lane 2 - 10).
(B) of peroxidase for IR 69625 A (lane 1) and their nine F_1 hybrids (lane 2 - 10).
(C) of peroxidase for IR 70368 A (lane 1) and their nine F_1 hybrids (lane 2 - 10).

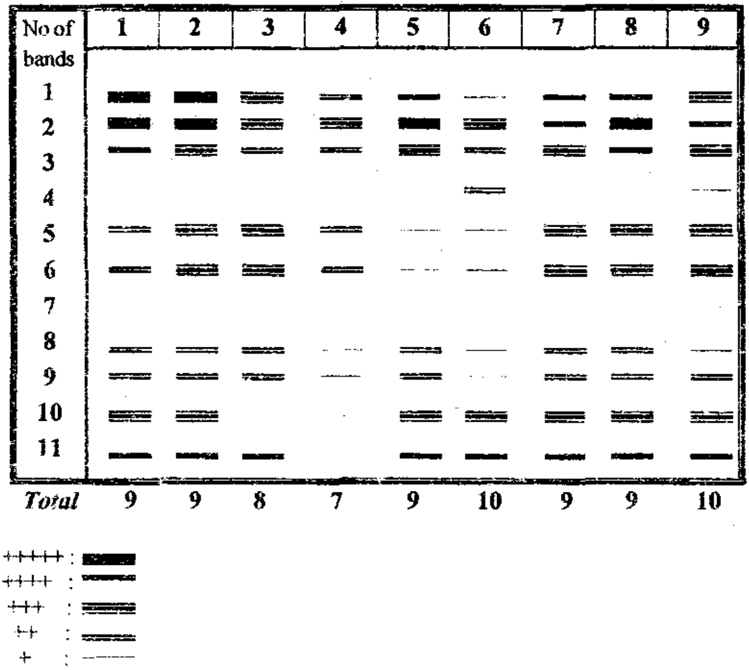


Fig. (2) Isozyme diagram of peroxidase for the nine testers.

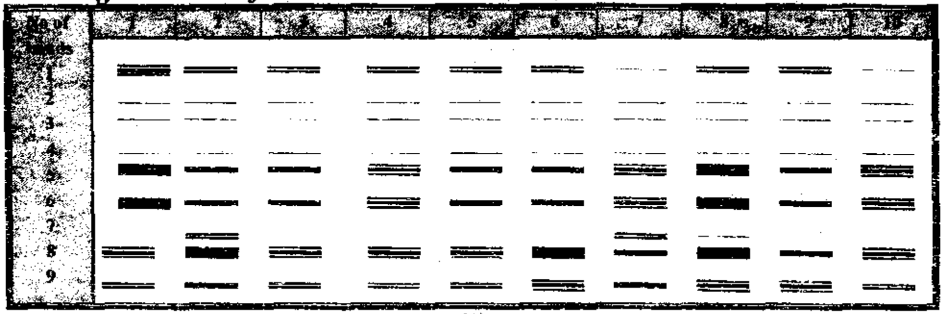
With respect to the esterase zymogram, a complex of sub-patterns were observed among the different tested genotypes. Description of esterase isozyme patterns of the three CMS lines and the nine testers showed that IR 58025 A and IR 69625 A exhibited the same number of bands (eight bands) as shown in (Figure 3 A and B), the band No.7 was absent in both lines. But the IR 70368 A line showed nine bands with different activity (Figure 3 C). On the other hand, all testers were differed in number of bands (ranged from seven to nine) and the activity (Figure 4).

2. Total Soluble Protein Analysis:

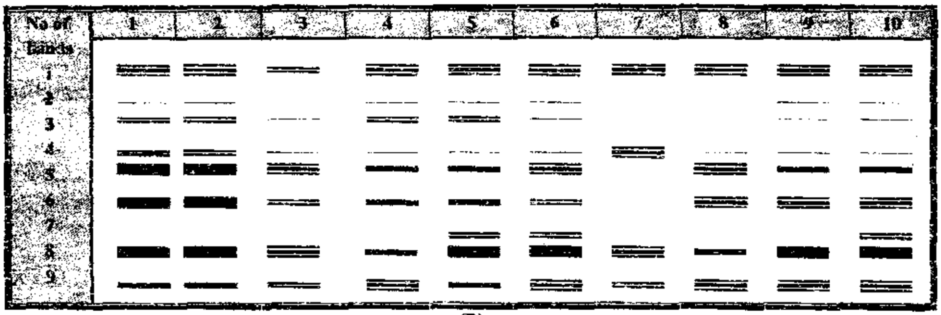
Total soluble proteins were detected using Coomassi Brilliant blue-R250 for the CMS lines IR 58025 A and IR 69625 A and their hybrids as shown in (Table 7). The first CMS line exhibited a total number of 13 bands, most of them were present in the tested entries, while their intensity varied in most cases.

For the CMS line IR 69625 A and their hybrids, most of bands were present in the ten tested entries and their intensity varied from case to another, except band No. 2 which was absent in the crosses IR 69625 A x IRGA 370-42-1-1F-C-1 and IR 69625 A x IR 69312-14-2-2-2.

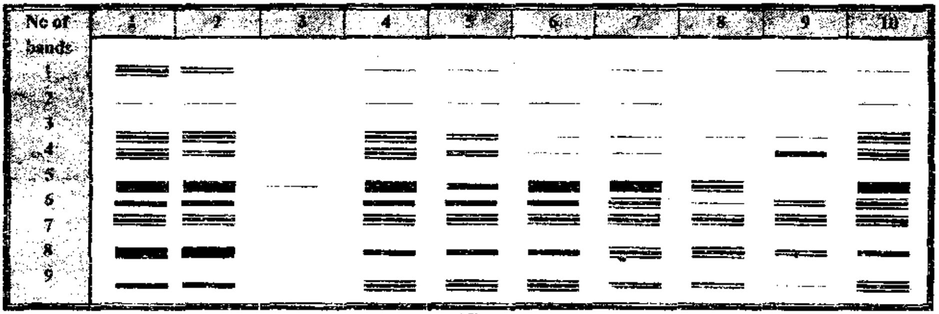
Generally some bands were missing in the CMS lines while present in most of the hybrids ,as well as, the testers. These bands might be derived from the testers. The bands were missing in both the CMS and the testers may present in the F_1 hybrids. Such observation as in the case of IR 58025 A x Giza 181 might have some relation with restorer genes, since Giza 181 is a good restorer. The reverse also was true in other cases, where the band was present in both CMS and tester while it was absent in their F_1' s. Thus, in general terms,



(A)



(B)



(C)

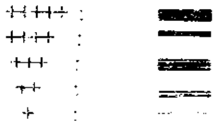


Fig. (3) Isozyme diagrams:

(A) of esterase for IR 58025 A (lane 1) and their nine F_1 hybrids (lane 2 - 10).
(B) of esterase for IR 69625 A (lane 1) and their nine F_1 hybrids (lane 2 - 10).
(C) of esterase for IR 70368 A (lane 1) and their nine F_1 hybrids (lane 2 - 10).

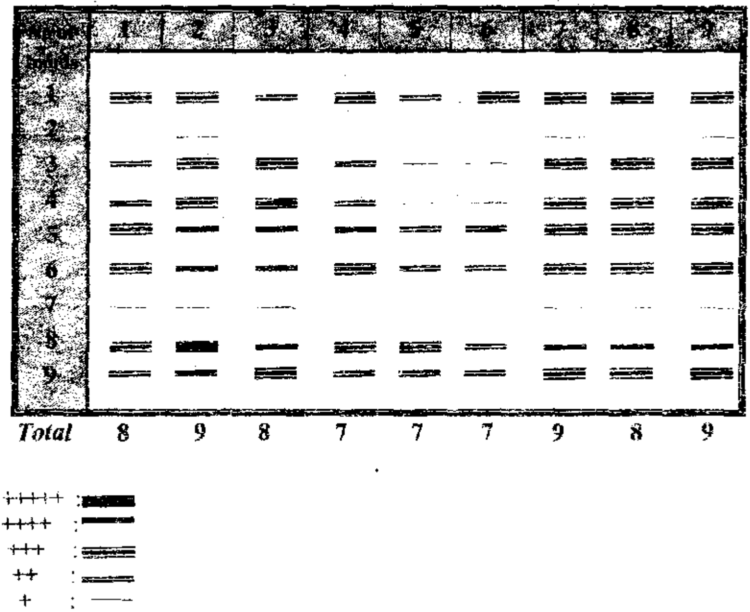


Fig. (4) Isozyme diagram of esterase for nine testers.

Table (7): Description of total soluble protein of IR 70368 A with their F_1 hybrids and nine testers .

Genotype / Bands No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
2	-	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
3	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	++	+++	+++	-
4	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
5	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
6	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+
7	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
8	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
9	-	-	+++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
10	+++	-	++++	++	++	++	+	++	++	++	++	++	++	++	++	++	++	++	++
11	+++	++++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	++++
12	+++	++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	++	+++	+++	+++	++
13	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	++	+++	+++	+++	+++

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

1- IR 70368 A	11- Giza 175
2- IR 70368 A x Giza 175	12- Giza 178
3- IR 70368 A x Giza 178	13- Giza 181
4- IR 70368 A x Giza 181	14- Giza 182
5- IR 70368 A x Giza 182	15- IRGA 370-42-1-1F-C-1
6- IR 70368 A x IRGA 370-42-1-1F-C-1	16- WAB 326-B-B-15
7- IR 70368 A x WAB 326-B-B-15	17- CT9685-14-M-1-2-3-2P-M-1
8- IR 70368 A x CT9685-14-M-1-2-3-2P-M-1	18- IET 12515
9- IR 70368 A x IET 12515	19- IR 69312-14-2-2-2
10-IR 70368 A x IR 69312-14-2-2-2	

there was poor correlation between total soluble proteins and restoring ability despite the polymorphism detected.

3. DNA Marker Analysis:

Using RAPD marker RBL.4 for PCR and subsequent separation of PCR products using 1.5% Agarose gel electrophoresis, a total of 14 amplified fragments were detected for the CMS line IR 58025 A along with nine testers and their respective hybrids (Fig. 5 A). The amplified fragments varied in their intensity and mobility, 50 bp ladder DNA was used as a size marker as shown in the first lane. Some of the amplified fragments were monomorphic and appeared in most of the tested materials. Bands were numbered on the basis of their respective sizes.

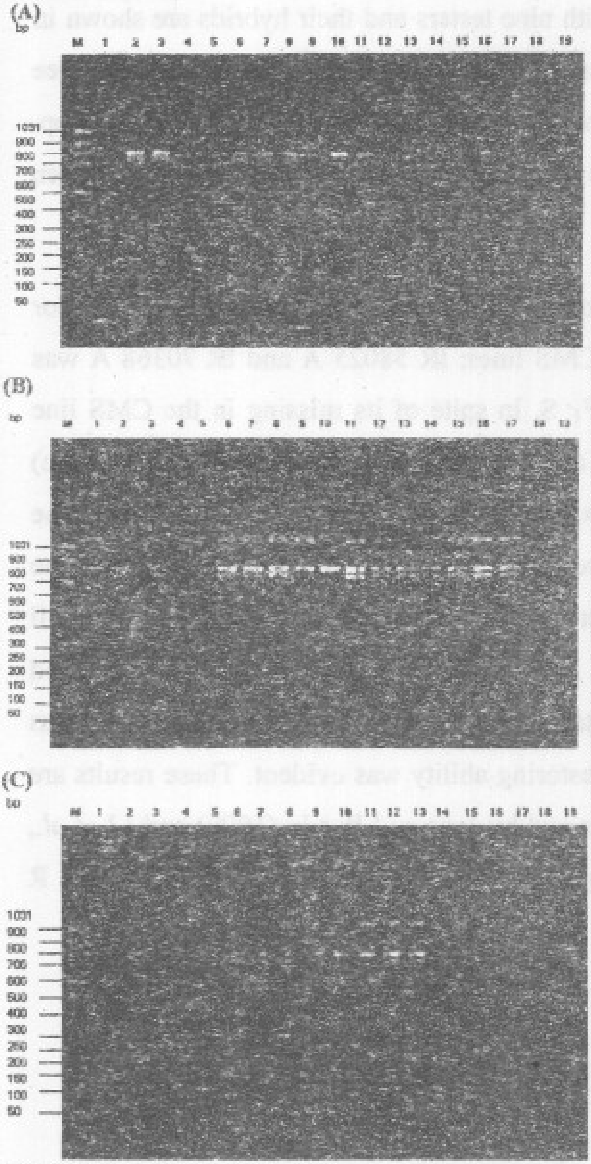
The first four bands had fragment sizes larger than 1031 bp, the largest band in the 50 bp ladder used. The largest fragment with approximately size of 1420 bp was present in eight genotypes and absent in 11 cases. The second band with size of 1350 bp appeared in 12 genotypes and their hybrids with the CMS line IR 58025 A. Band No. five with a size of 925 bp appeared in ten cases and was absent in nine lanes. The size of the remained 12 fragments ranged between 1155bp and 217bp. They varied in their intensity and presence or absence in the CMS line IR 58025 A and their F1,s as shown in (figure 5 A).

The sixth band with size of 816 bp was present in all samples, while it was very faint in case of CMS line IR 58025 A.

As for CMS line IR 69625 A along with the nine testers and their respective hybrids (Figure 5 B), the first four bands also had fragment sizes larger than 1031 bp. The largest fragment with band size of 1420 bp was present in 13 genotypes and absent in six.

The CMS line IR 70368 A with nine testers and their hybrids are shown in (Figure 5 C), 14 amplified fragments were detected for the CMS line with three respective hybrids. The first four bands had fragments were larger than 1031 bp. The largest fragment with approximate size of 1420 bp was present in ten genotypes and absent in nine.

From DNA studies, a level of molecular polymorphism was detected. For example, band No. 7 in the two CMS lines; IR 58025 A and IR 70368 A was found in many testers and their F_1' S, in spite of its missing in the CMS line itself (Figure 5 C). Figure 6 a, b and c are diagrams for (Figure 5 a, b and c) respectively. Therefore, further experiments could be applied for studying the correlation of this band with the restoring ability of CMS lines. Also, the same trend of polymorphism was detected in the case of total soluble proteins in all CMS lines, testers and their F_1'S . Most of the testers are considered to be good restorers. In case of the rest of detected bands, a quite poor correlation between the detected polymorphism and restoring ability was evident. These results are in correspondence with those obtained by Jena and Pandy (1999) and Li *et al.*, (2000), who found few polymorphic DNA profiles between A (CMS) and R (restorer) lines.



1(A): IR 58025 A
(B): IR 69625 A
(C): IR 70368 A

2 (A, B and C): Giza 175

3 (A) IR 58025 A x Giza 175
(B) IR 69625 A x Giza 175
(C) IR 70368 A x Giza 175

4 (A, B and C): Giza 178

5 (A) IR 58025 A x Giza 178
(B) IR 69625 A x Giza 178
(C) IR 70368 A x Giza 178

6: (A, B and C) Giza 181

7: (A) IR 58025 A x Giza 181
(B) IR 69625 A x Giza 181
(C) IR 70368 A x Giza 181

8: (A, B and C) Giza 182

9: (A) IR 58025 A x Giza 182
(B) IR 69625 A x Giza 182
(C) IR 70368 A x Giza 182

10: (A, B and C) IRGA 370-42-1-1F-C-1

11: (A) IR 58025 A x IRGA 370-42-1-1F-C-1
(B) IR 69625 A x IRGA 370-42-1-1F-C-1
(C) IR 70368 A x IRGA 370-42-1-1F-C-1

12: (A, B and C) WAB 326-B-B-15

13: (A) IR 58025 A x WAB 326-B-B-15
(B) IR 69625 A x WAB 326-B-B-15
(C) IR 70368 A x WAB 326-B-B-15

14: (A, B and C) CT9685-14-M-1-2-3-2P-M-1

15: (A) IR 58025 A x CT9685-14-M-1-2-3-2P-M-1
(B) IR 69625 A x CT9685-14-M-1-2-3-2P-M-1
(C) IR 70368 A x CT9685-14-M-1-2-3-2P-M-1

16: (A, B and C) IET 12515

17: (A) IR 58025 A x IET 12515
(B) IR 69625 A x IET 12515
(C) IR 70368 A x IET 12515

18: (A, B and C) IR 69312-14-2-2-2

19: (A) IR 58025 A x IR 69312-14-2-2-2
(B) IR 69625 A x IR 69312-14-2-2-2
(C) IR 70368 A x IR 69312-14-2-2-2

Fig. (5): DNA polymorphism:

(A) for CMS line IR 58025 A and their respective F_1' s detected by RAPD marker RBL.4.
(B) for CMS line IR 69625 A and their respective F_1' s detected by RAPD marker RBL.4.
(C) for CMS line IR 70368 A and their respective F_1' s detected by RAPD marker RBL.4.

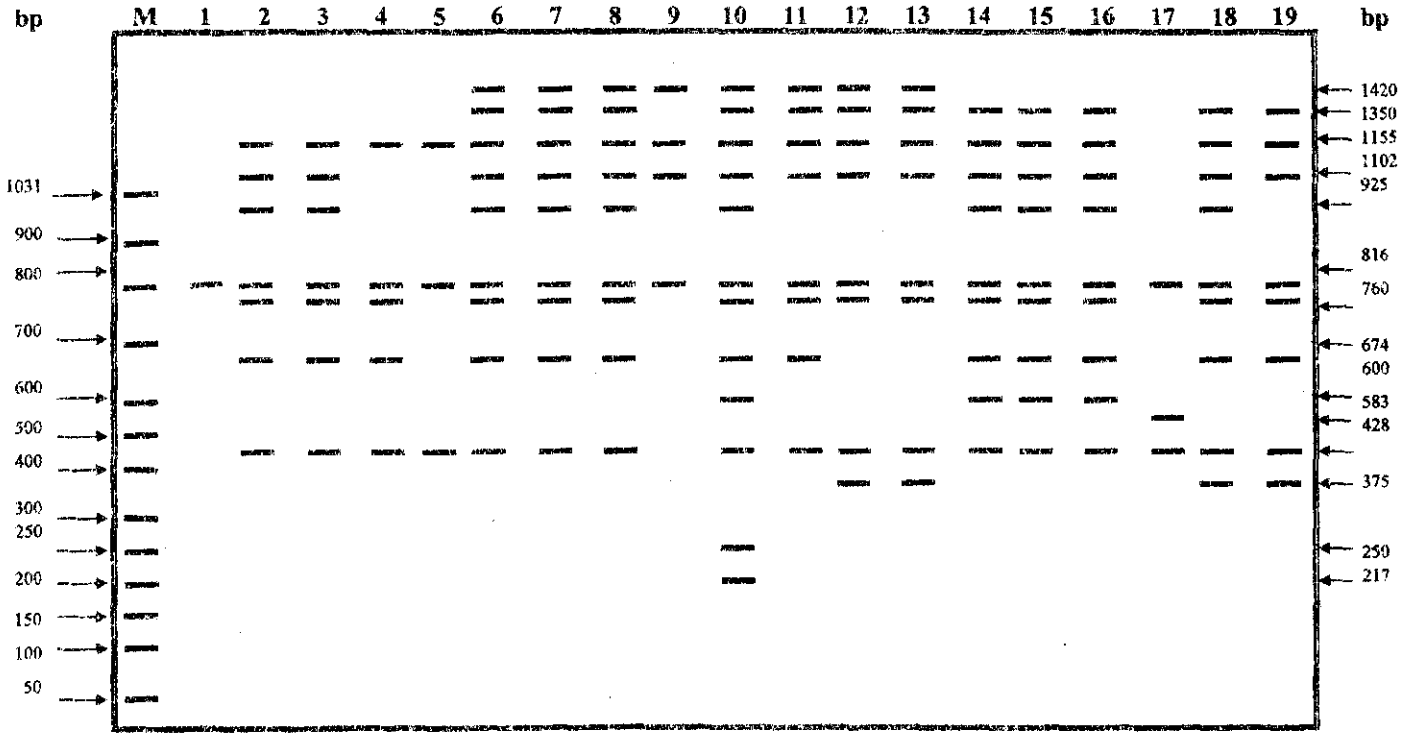

bp
M 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
bp
1031
900
800
700
600
500
400
300
250
200
150
100
50
1420
1350
1155
1102
925
816
760
674
600
583
428
375
250
217

Fig. (6 a)

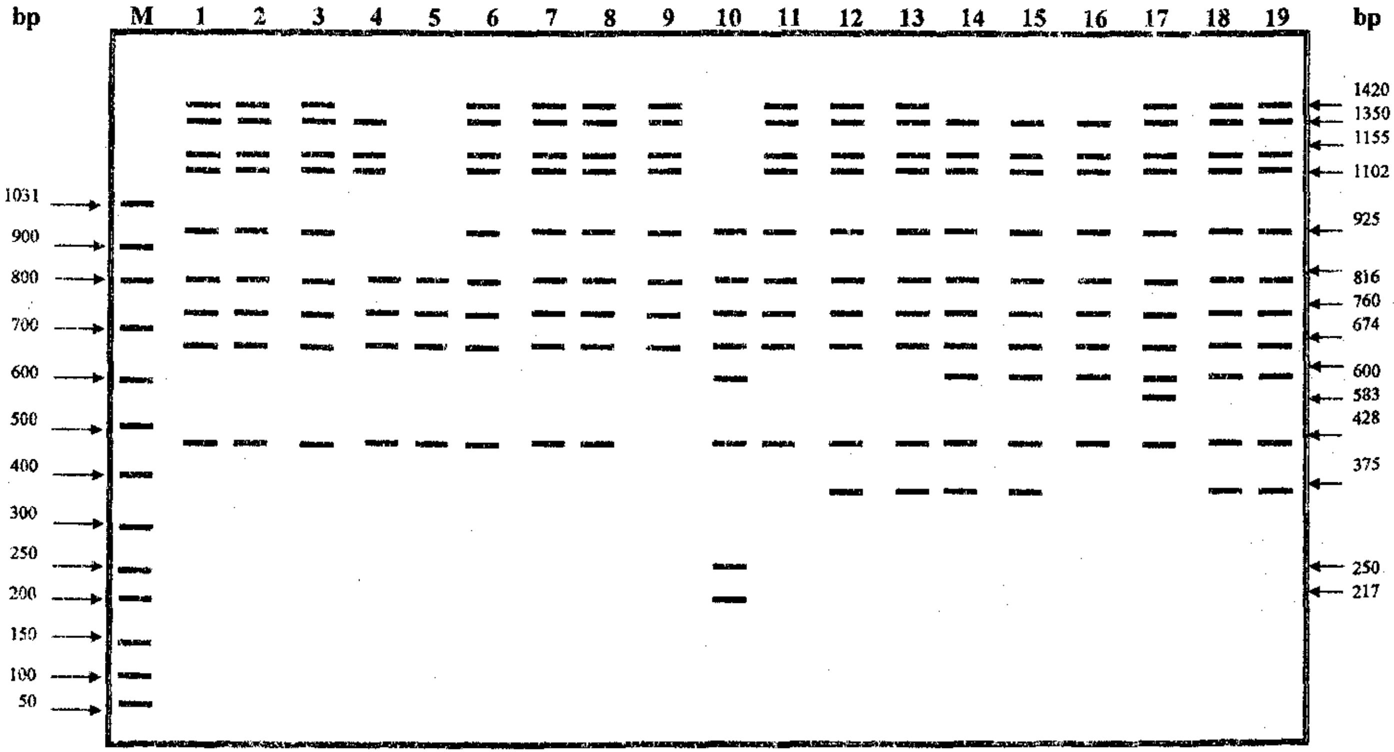

bp
M
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
bp
1031
900
800
700
600
500
400
300
250
200
150
100
50
1420
1350
1155
1102
925
816
760
674
600
583
428
375
250
217

Fig. (6 b)

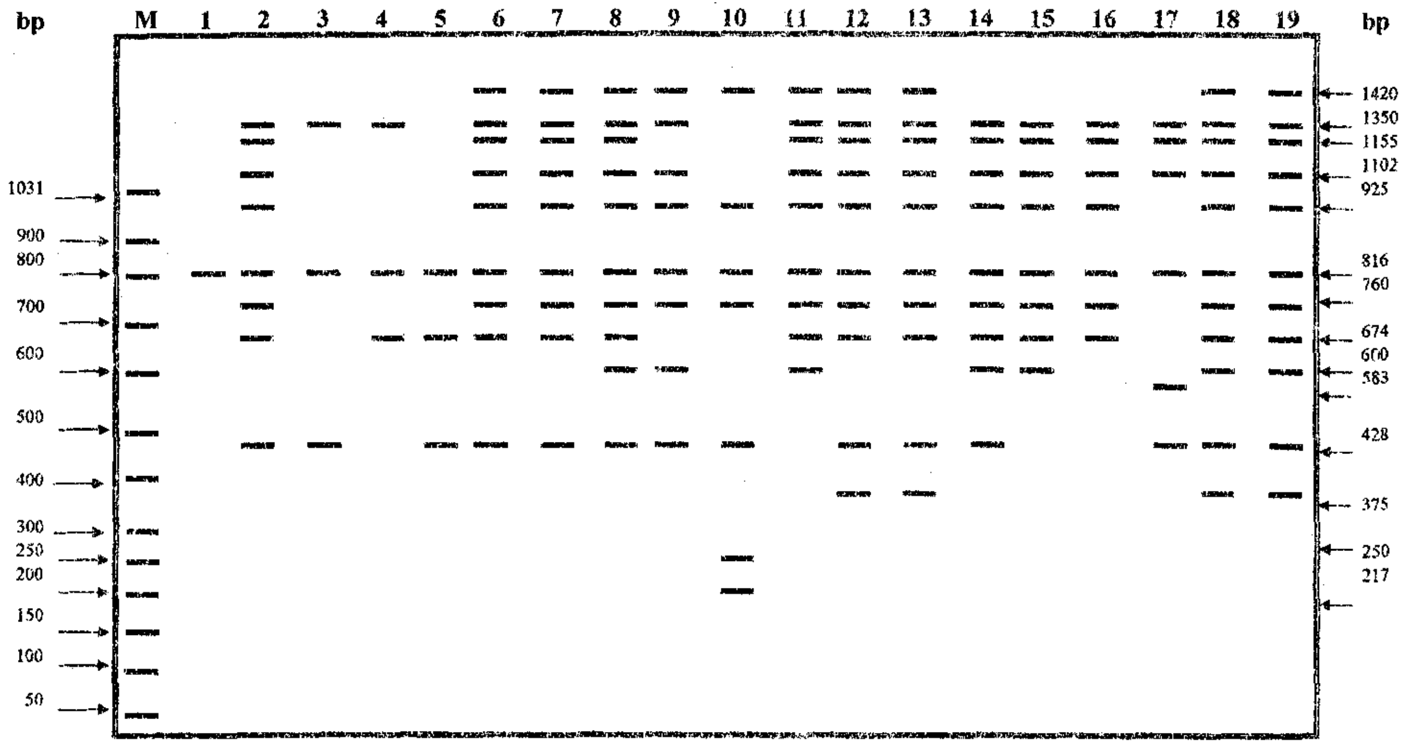

bp
M
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
bp
1031
900
800
700
600
500
400
300
250
200
150
100
50
1420
1350
1155
1102
925
816
760
674
600
583
428
375
250
217

Fig. (6 c)

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الملخص العربى

دراسات مورفولوجية ووراثية جزيئية على المقدرة على إعادة الخصوبة لسلالات الأرز العقيمة ذكرياً

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أستخدم في هذه الدراسة ثلاث سلالات أرز عقيمة ذكريا (CMS) وهي IR 58025 A ، IR 69625 A و IR 70368 A وتم أختبارها لبعض الصفات المورفولوجية والبيوكيمائية والجزيئية بواسطة تسعة أصناف مختلفة من الأرز. وقد استخدم نموذج line x tester بين الثلاث سلالات العقيمة ذكرياً (lines) والتسعة أصناف (testers) وذلك للحصول على ٢٧ هجين جيل أول (F_1's) وتم حصاد كل هجين منفصل عن الأخر.

أظهرت النتائج أن السلالات الأبوية و الهجن بها اختلافات معنوية واضحة. فكان اتجاه الخلط سلبى فى بعض الصفات مثل ايام الطرد، و كان الخلط ايجابى فى كثير من الصفات الأخرى. وتشير النتائج إلى أن السلالة IR 69625 A أعطت قيم مرغوبة لصفة عدد أيام التزهير وعدد الفروع فى النبات والمحصول فى النبات بينما أعطت السلالة IR 70368 A قيم مرغوبة فى صفة طول النبات ، طول السنبلة ، وزن القش ، دليل الحصاد ووزن الـ ١٠٠٠ حبة وأخيرا أعطت السلالة IR58025 A قيم غير مرغوبة فى معظم الصفات المدروسة تحت الظروف المصرية. وبالنسبة للقدرة العامة على التآلف أظهرت السلالة IK 69625 A أحسن قدرة تآلف خاصة بالنسبة لصفة محصول الحبوب/للنبات، بينما سجلت السلالة IR 58025 A

والسلالة IR 70368 A اختلافات معنوية ومعنوية جدا على التوالى بالنسبة لصفة وزن القش. وبالنسبة لتأثيرات القدرة الخاصة على التآلف سجل الهجين IR 58025 A x IRGA 370-42-1-1f-c1 والهجين IR 69625 A x Giza 175 قيما ايجابية معنوية بالنسبة لعدد الأشطاء.

أوضحت النتائج أن هناك إختلاف فى عدد و شدة بعض الحزم بين التراكيب الوراثية المختلفة بالنسبة لنشاط إنزيم البيروكسيديز، بعض هذه الحزم كان يميز السلالات العقيمة ذكريا. كما أوضحت النتائج بالنسبة لنشاط إنزيم الأستيريز أن هناك اختلافات معقدة فى الحزم بين التراكيب الوراثية المختلفة. كما تم استكشاف البروتينات الكلية الذائبة فى السلالات العقيمة ذكريا IR 58025 A و IR 69625 A و هجنها . بشكل عام فإن تحليل البروتينات الذائبة الكلية اظهر وجود بعض الحزم فى اغلب الهجن والأصناف تحت الدراسة فى حين كانت غائبة فى حالة السلالة العقيمة ذكرياً واغلب الظن أن هذه الحزم هى نفسها الموجودة فى الاباء على إعتبار أنها غير موجودة فى السلالة العقيمة ذكريا. ويمكن الربط بين مثل تلك الحزم وبين القدرة على إعادة الخصوبة. كما أظهرت الدراسة بمعلمات الـ DNA الجزيئية درجة عالية من الإختلاف بإستخدام المعلم الجزيئى RBL.4 إذ لوحظت حزم محددة في الهجن وكذلك فى الأصناف تحت الدراسة فى حين كانت تلك الحزم غير موجودة فى السلالة العقيمة ذكريا. إلا أن الإرتباط بين تلك الإختلافات والقدرة على إعادة الخصوبة كان ضعيفا إلى حد كبير ويرجع ذلك إلى إستخدام معلمات الـ RAPD وهى معلمات عادة ما تكون غير متخصصة للصفة محل الدراسة وهذا يحتاج مزيدا من الدراسة.