



USING LINE × TESTER ANALYSIS TO DEVELOP NEW SOURCE OF CYTOPLASMIC MALE STERILE LINE IN HYBRID RICE

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Abstract

Five cytoplasmic male sterile lines with their maintainer of different sources of cytoplasmic male sterile were used to make possible combination by using line × tester model to develop new source of cytoplasmic male sterile, this experiment was conducted at experimental farm of Rice Research Section, Sakha, Kafrelsheikh during and 2014 and 2015 season in RCB design with three replication. The data were recorded on some anther, pollen grains, morphological and yield characters, the results showed that, most of the studied characters exhibited differences and highly significant especially for anther shape and color of pollen grains as well as, panicle exertion % seed set % and grain yield per plant¹ there were ranged (86.33-100), (86.04-100), (71.57-87.04), (10.23-18.07) and (12.03-16.03) for normal anther and color of pollen grains as well as, panicle exertion % seed set % and grain yield per plant¹, whereas, testers were recorded 0.01 for anther shape and weight color of pollen grains (97.00-99.67), (92.05-95.13) and (38.10-45.33) for panicle exertion %, seed set % and grain yield per plant¹ whereas the hybrid combinations were ranged (71.43-100), (73.30-100), (57.37-90.87), (7.03-18.07) and (11.03-18.12) for these characters. *SCA* was higher than *GCA* for all the studied characters indicating the dominance gene action played importance role in the genetic control for these characters in develop new source of cytoplasmic male sterile lines in hybrid rice breeding program.

Key words : Panicle exertion, pollen grains, *SCA*, *GCA*, hybrid rice, pollen grains.

Introduction

Rice is the world leading cereal crop for human utilization, with cultivated area of almost 150 million ha and a total production of almost 600 million mega grams annually (Khush, 2005). The world population is expected to reach 8 billion by 2030 and rice production must be increased by 50% in order to meet the growing demand for the world (Khush and Brar, 2002). Hybrid rice has proven to be an effective and economical way to increase rice production output. It is easy to obtain about 15-20% higher yield just by growing hybrid rice instead of the common varieties (Virmani *et al.*, 1997) and in China gave more than 30% yield advance over conventional pure rice production by nearly 200 million tons from 1976 to 1991 (Yuan, 1998). China is the first country to exploit commercially heterosis in rice. Hybrid rice technology was successfully developed in 1976 using male sterility-fertility restoration system (Anonymous, 1977). However,

the success story of commercial hybrid rice cultivation in China, India, Vietnam, Philippines and Bangladesh has encouraged Egypt to adopt this technology (Bastawisi *et al.*, 2003). Combining ability is a measure of gene action, the general combining ability (*GCA*) effects involve additive gene effects, whereas, specific combining ability (*SCA*) represent only non additive gene action. However, the parents with good (*GCA*) effect could be used to obtain hybrids with strong heterosis (Yan *et al.*, 2000). Three major characters *viz*, anther shape, color and panicle exertion as well as shape and color of pollen grains are related to cytoplasmic male sterile trait which using selection in early generation for sterile plant type. Therefore, this paper aimed to, study the magnitude of genotypic variation for the different sources of cytoplasmic male sterile, the magnitude of both general and specific combining abilities for maintaining ability and study the gene expression for the studied characters.

Materials and Methods

The present investigation was carried out at the farm of Rice Research and Training Center (RRTC), Sakha, Kafr El-Sheikh, Egypt, during the two successive growing seasons of 2014 and 2015. The genetic materials used in this investigation involved five CMS lines of rice obtained from different sterile sources of Wild Abortive, Gambiaca and Kalinga. These lines were, IR69625A, IR70368A, IR58025A, K17A and G46A which used as female parents. In addition five maintainer for these the CMS lines *viz.*, IR69625B, IR70368B, IR58025B, K17B and G46B were used as "Testers". These materials (CMS lines and testers) were planted during 2014 in different sowing dates to get good synchronization of flowering to make the crosses among the parental lines to obtain hybrid seeds. During 2015 seasons, the parents and their F_1 crosses were sown in the nursery on May 1st and seedlings were transplanted after 30 days. The parents and their F_1 crosses were grown in a Randomized Complete Block Design (RCBD) with three replications. Each replicate consisted of one row of F_1 cross between two rows of each parental lines with barrier isolation among each male parent, the row measured five meters long, 20 cm apart and contained 25 hills. All agricultural practices were made according to rice recommendations (RRTC, 2013). Data were recorded on 10 randomly selected plants from each replication and mean values were used for statistical analysis. Observation were recorded on the following characters *viz.*, up normal anther, pollen grains color, panicle exertion %, seed set % and grain yield plant⁻¹.

Statistical analysis

The data were subjected to analysis of variances for a randomized complete blocks design as suggested by Panse and Sukhatme (1954) and the analysis of variance for line x tester crossing design (Kempthorne, 1957). Some important genetic parameters such as additive variance, non additive variance, broad sense heritability (h^2_b) and narrow sense heritability (h^2_n) were also estimated according to Falconar and Mackey (1996).

Results and Discussion

Mean performance

Mean performance of lines, testers and their hybrids in table 1. There were significant differences among the genotype values for all studied characters, the highest values for up normal anther were recorded of CMS line IR69625A (100.00), while, the crosses; (IR69625A × G46B and G46A × IR69625B) indicating to these lines, it is complete sterile and could be used in backcross method

to develop new CMS lines. In contrast the lowest values for up normal anther were recorded of CMS line K17A, testers IR69625B, IR70368 B, IR 58025B and G46B and the cross K17A × IR58025B (71.43, 0.01, 0.01, 0.01, 0.01 and 71.43), respectively. Indicating to CMS line with their cross were unstable for sterility, while, the maintainer lines were high stable for self pollinated for color of pollen grains, CMS lines IR69625A and G46A, the crosses of IR69625A × IR70368B, IR69625A × IR58025B, IR69625A × G46B, IR70368A × IR58025B, IR58025A × G46B and G46A × K17B respectively, were recorded the highest values 100.0 unstand pollen grains indicating to these materials are considered as good source to sterile plant , but, the lowest one were recorded of line K17A, and the cross (K17A × IR58025B), these result were confirmed with the data which recorded on anther shape . Concerning to the panicle exertion, the highest values for panicle exertion were observed with CMS line IR 69625A (87.04%), tester IR69625B (99.67) and the cross G46A × IR 69625B (80.69%). On the other hand, the lowest values for panicle exertion % were observed with CMS lines K17A (71.57%), tester IR58025B (97%) and the cross IR69625A × G46B (57.37%) respectively, indicating to these lines unstable for sterility and require more time to reach high stable value, this result similar to Swati and Ramesh (2004). For seed set %, the highest values for seed set % were recorded of CMS line IR69625A (18.07%), the tester (>90%) and the crosses IR 69625A × IR69625B and IR58025A × IR58025B respectively, but the lowest values for seed set % were recorded with the line IR K17A and the cross IR70368 x IR58025B (10.23 and 7.03 %) respectively. For grain yield plant⁻¹, the parents IR69625A, IR69625B and the crosses IR58025A × G46B gave desirable mean values (16.03, 45.33 and 18.12 gm), respectively. While, the lowest values for grain yield were recorded of CMS line K17A, the tester K17B and the cross K17A × K17B (11.03, 38.10 and 11.03 gm plant⁻¹), respectively. The results of the general mean, for the lines, testers and F_1 crosses of the studied characters, indicated that, up normal anther, pollen grain color, panicle exertion %, seed set % and grain yield were highly affected by the maternal effect, this means that, the selection should be direct to the plant type of sterile plant, which affected by the maternal effect to develop new CMS lines, but for the characters, which affected by the male effect should be direct selection to the fertile plant to develop new maintainer line.

Analysis of variances

The analysis of variance revealed significant differences among genotypes, crosses, lines, testers and

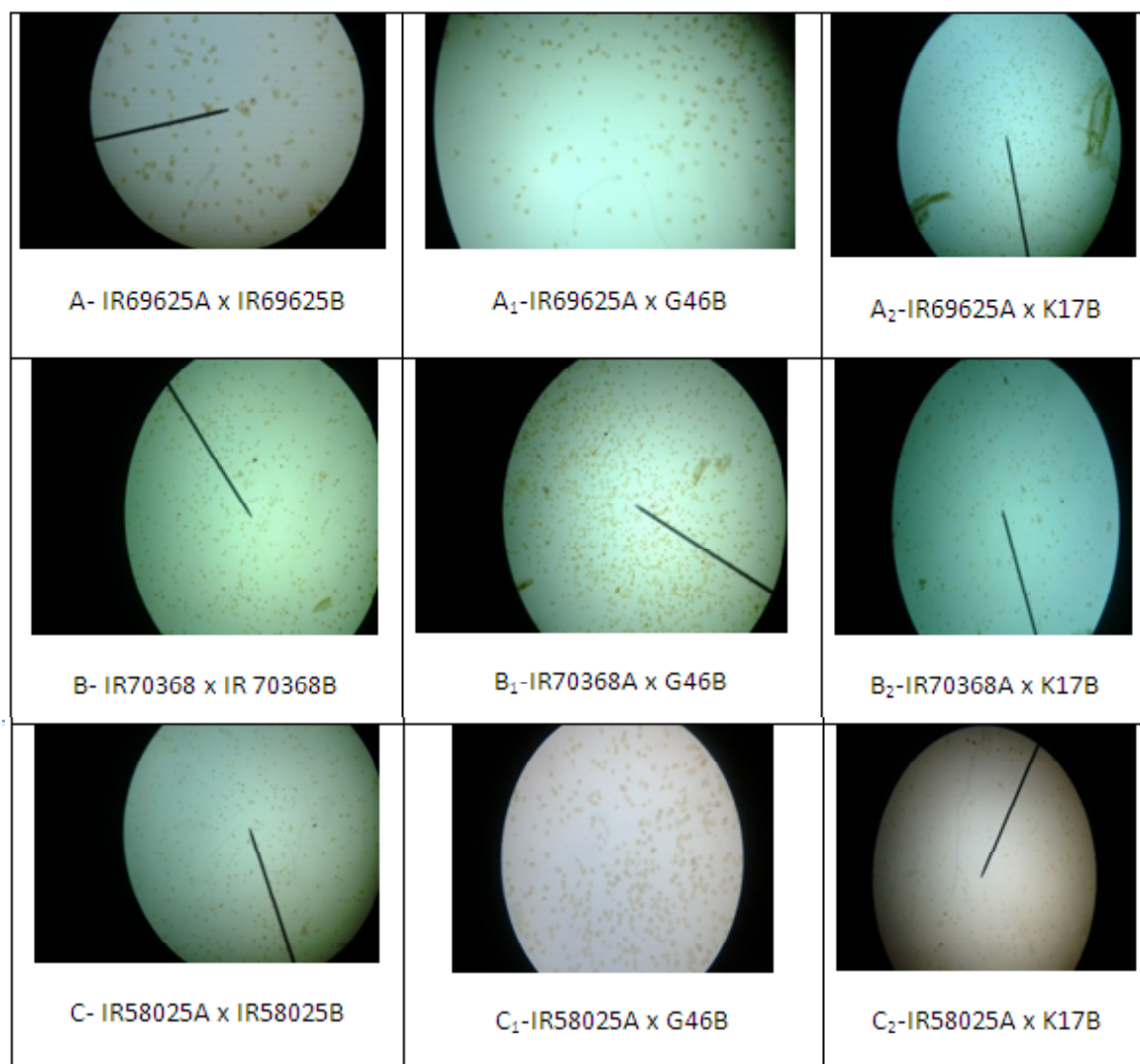


Fig. 1 : Showed complete sterility for different combinations comparing to CMS lines with their maintainer.

line × tester interactions for all studied characters (table 2). These results indicated that genotypic differences among entries were present. Mean square values of parents and crosses were found to be highly significant for studied characters. These results could be used as an indication to average heterosis over all crosses and therefore could be used through hybrid breeding technology to improve such traits (Wilfred and Palanisamy, 1989). These differences between mean square values for lines x testers were highly significant for all studied characters, indicating that non additive (dominance or epistasis) genetic variance were of great importance in the inheritance of these characters. These results revealed that dominance and dominance × dominance gene action were important in the inheritance of these characters. Similar results were found by Sarathe and Singh (1986). The mean square of *SCA* was higher than those of the *GCA* variances for these characters indicated

preponderance of non-additive gene action in the inheritance of these traits. This was further supported by low magnitude of *MSgca/MSsca* ratios (table 2). It suggested greater importance of non-additive gene action in its expression and indicated very good prospect for the exploitation of non-additive genetic variation for these traits through hybrid breeding (Ramalingam *et al.*, 1997; Annadurai and Nadarajan, 2001).

General Combining Ability

The General Combining Ability (*GCA*) effects of lines (table 3) revealed significant differences among them. Among the five CMS lines tested, IR69625A was a good combiner for up normal anther % and wight color of pollen grains, whereas IR58025A was recorded good combiner for seed set % and grain yield per plant⁻¹ and the line IR 70368 A was the best combiner for panicle exsertion%. Similar findings have been reported by Surek and Korkut

Table 1 : The mean performances of all genotypes for some characters during 2015 season.

Genotypes	Up normal anther (%)	Weight color of pollen grain	Panicle exsertion (%)	Seed set% unclosed panicle	Grain yield per plant ¹
CMS Lines	-	-	-	-	-
IR69625	100.00	100.00	87.04	18.07	16.03
AIR70368	93.31	93.34	82.63	12.10	14.07
AIR 58025A	93.41	86.62	77.67	16.10	15.03
K17A	86.33	86.04	71.57	10.23	11.03
G46A	86.60	99.67	73.47	11.07	12.03
Mean	89.33	93.13	78.476	13.51	13.64
(Testers)	-	-	-	-	-
IR69625B	0.01	0.01	-99.67	-95.13	45.33
IR70368 B	0.01	0.01	99.33	93.22	41.20
IR 58025B	0.01	0.01	97.00	92.41	43.61
K17B	0.04	0.01	98.00	92.05	38.10
G46B	0.01	0.01	99.33	94.35	44.19
Mean	0.016	0.01	98.666	93.43	42.49
Hybrid combinations :	-	-	-	-	-
IR69625A * IR69625B	100.00	99.67	81.33	18.07	16.03
IR69625A * IR70368 B	100.00	100.00	80.63	9.17	15.03
IR69625A * IR 58025B	100.00	100.00	73.87	11.37	14.00
IR69625A * K17B	86.72	86.69	71.37	8.60	13.03
IR69625A * G46B	100.00	100.00	57.37	15.37	16.00
IR70368A IR69625B	95.10	92.33	90.87	11.10	15.05
IR70368A * IR70368 B	93.31	93.34	90.67	12.10	14.07
IR70368A * IR 58025B	100.00	100.00	83.53	7.10	13.00
IR70368A * K17B	86.65	86.64	75.23	7.03	12.00
IR70368A * G46B	93.32	73.41	90.07	10.53	16.00
IR 58025A * IR69625B	99.67	99.83	77.73	7.07	13.00
IR 58025A * IR70368 B	100.00	99.83	75.27	14.30	17.35
IR 58025A * IR 58025B	93.41	86.62	70.57	16.10	15.03
IR 58025A * K17B	86.65	73.54	72.97	11.10	15.08
IR 58025A * G46B	100.00	100.00	70.17	15.33	18.12
K17A * IR69625B	80.03	85.13	77.70	9.94	15.20
K17A * IR70368 B	73.31	80.10	73.60	7.23	13.00
K17A * IR 58025B	71.43	64.33	68.37	9.13	13.07
K17A * K17B	86.33	86.60	63.57	10.23	11.03
K17A * G46B	80.10	66.57	63.43	8.50	16.00
G46A * IR69625B	100.00	93.44	76.33	10.07	14.00
G46A * IR70368 B	80.10	73.30	75.87	9.91	13.57
G46A * IR 58025B	100.00	93.41	75.27	10.23	12.50
G46A * K17B	86.60	100.00	68.07	10.13	14.00
G46A * G46B	100.00	100.00	68.40	11.07	12.03
Mean	91.19	89.39	74.89	10.83	14.29
G. Mean	77.89	77.16	78.80	23.01	18.22

(2002). The testers, IR58025B were the best general combiner for up normal % and wight color of pollen grains whereas, the testers IR69625B were recorded best general combiner for panicle exsertion %, G46B was recorded best general combiner for seed set and grain yield per plant⁻¹ and could be used these parents in

breeding programs to developing new CMS lines with stable to sterility trait.

Specific Combining Ability

Estimates of the specific combining ability effects (SCA) of the hybrid combinations are given in table 4.

Table 2 : Estimates of the mean square of line x tester analysis for some characters during 2015 season.

d. f	S. O. V	Character				
		Up normal anther(%)	Wight color of pollen grain	P. exertion (%)	Seed set % Unclosed panicle	Grain yield per plant ¹
Reps	2	0.02ns	0.03ns	1.05ns	0.01ns	0.01ns
Genotypes (G)	34	3367.62**	3502.61***	369.38**	2578.20**	313.92**
Parents (P)	9	6784.03**	6716.51**	396.17**	5340.39**	710.17**
P vs. Cr	1	46366.48**	43708.69**	4011.17**	38964.80**	4065.89**
Crosses (Cr)	24	294.85**	622.14**	207.59**	26.27**	9.00**
Lines (L)	4	1173.81**	1865.62**	625.04**	44.40**	14.73**
Testers (T)	4	153.51**	439.29**	376.74**	15.07**	15.78**
Lx T	16	110.45**	356.99**	60.94**	24.54**	5.87**
Error	68	0.01	0.03	2.94	0.01	0.00
GCA		4.85	6.98	3.86	0.09	0.16
SCA		36.81	118.99	19.33	8.17	1.96
GCA/SCA		0.011	0.005	0.005	0.0003	0.004

* and ** Significant at 0.05 and 0.01 levels, respectively.

Table 3 : Estimates of GCA effects (g.) of the CMS lines and maintainer lines (Testers) for some characters during 2015 season.

Genotypes	Up normal anther %	Wight color of pollen grains	Panicle exertion (%)	Seed set (%)	Grain yield per plant ¹
CMS Lines	-	-	-	-	-
IR69625A	6.15	9.47	-1.97	1.68	0.53
IR70368A	2.48	1.35	11.18	-1.25	-0.26
IR 58025A	4.75	4.17	-1.54	1.94	1.42
K17A	-15.54	-19.24	-5.55	-1.82	-0.62
G46A	2.15	4.23	-2.10	-0.54	-1.06
(Testers)	-	-	-	-	-
IR69625B	2.05	2.12	5.90	0.41	0.36
IR70368 B	-1.84	1.52	4.31	-0.28	0.31
IR 58025B	3.49	5.24	-0.56	-0.04	-0.76
K17B	-4.51	-9.09	-4.64	-1.41	-1.25
G46B	0.81	0.20	-5.00	1.32	1.34
L.S.D. for CMS lines	0.05	0.09	0.88	0.06	0.03
	0.01	0.12	1.17	0.09	0.05
L.S.D. for testers	0.05	0.09	0.88	0.06	0.03
	0.01	0.12	1.17	0.09	0.05

The cross combinations G46A × K17B was the best specific combination for up normal anther and wight color of pollen grains, the cross combination IR70368A × G46B were the best specific combiner for panicle exertion %. Concerning to seed set % the hybrid combination IR 69625A × IR 69625B was recorded the best specific combining ability for this characters, while, the hybrid cross G46A × K17B was recorded the best specific

combining ability for this characters. These results indicated that the possibility to exploit these combinations in developing hybrid rice combinations. It also indicated that both additive and non-additive types of gene action play important for the inheritance of these traits.

Genetic parameters and heritability

Estimation of genetic parameters and heritability are

Table 4 : Estimates of SCA effects (Sij) of hybrid combinations for some characters during 2015 season.

Genotypes	Up normal anther (%)	Wight color of pollen grains	Panicle exsertion (%)	Seed set (%)	Grain yield per plant ¹ (g)
Hybrid combinations :	-	-	-	-	-
IR69625A * IR69625B	0.60	0.27	2.51	5.13	0.84
IR69625A * IR70368 B	4.50	1.21	3.40	-3.05	-0.10
IR69625A * IR 58025B	-0.84	-2.51	1.52	-1.10	-0.05
IR69625A * K17B	-6.10	-1.49	3.10	-2.50	-0.52
IR69625A * G46B	1.84	2.53	-10.54	1.52	-0.16
IR70368A IR69625B	-0.63	1.06	-1.11	1.11	0.65
IR70368A * IR70368 B	1.48	2.67	0.27	2.81	-0.27
IR70368A * IR 58025B	2.82	5.61	-1.97	-2.42	-0.25
IR70368A * K17B	-2.50	6.59	-6.19	-1.12	-0.76
IR70368A * G46B	-1.17	-15.94	8.99	-0.36	0.63
IR 58025A * IR69625B	1.67	5.74	-1.51	-6.12	-3.08
IR 58025A * IR70368 B	5.90	6.34	-2.39	1.80	1.31
IR 58025A * IR 58025B	-6.03	-10.59	-2.20	3.36	0.08
IR 58025A * K17B	-4.78	-9.32	4.27	-0.26	0.62
IR 58025A * G46B	3.24	7.83	1.82	1.22	1.05
K17A * IR69625B	-6.26	-6.35	2.46	0.51	1.17
K17A * IR70368 B	-0.49	10.03	-0.05	-1.48	-0.97
K17A * IR 58025B	0.89	11.34	-0.39	0.17	0.17
K17A * K17B	2.21	-12.84	-1.11	2.63	-1.36
K17A * G46B	3.64	-2.18	-0.89	-1.83	0.99
G46A * IR69625	4.61	-0.71	-2.35	-0.63	0.41
BG46A * IR70368 B	-11.39	-20.25	-1.23	-0.08	0.03
G46A * IR 58025B	3.16	-3.86	3.04	-0.00	0.04
G46A * K17B	11.18	17.06	-0.07	1.26	2.03
G46A * G46B	-7.55	7.76	0.61	-0.54	-2.52
L.S.D.	0.05	0.13	0.20	0.15	0.08
	0.01	0.18	0.27	0.20	0.11

Table 5 : Estimates of genetic parameters and heritability in broad and narrow senses for the studied characters during 2015 season.

Genetic parameters and heritability	Up normal anther (%)	Wight color of pollen grains	Panicle exsertion (%)	Seed set (%) un-closed panicle	Grain yield per plant ¹ (g)
Additive variance (σ^2A)	9.70	13.95	7.71	0.09	0.16
Dominant variance (σ^2D)	36.81	118.98	19.33	8.17	22.85
Genotypic variance (σ^2G)	46.51	132.93	27.05	8.26	23.01
Environmental variance (σ^2E)	0.02	0.06	3.98	0.02	0.10
Phenotypic variance (σ^2P)	93.05	265.94	58.08	16.55	46.13
Broad sense heritability (h ² b%)	99.96	99.97	90.20	99.79	99.73
Narrow sense heritability (h ² n%)	20.85	10.49	25.73	1.09	0.69
Relative importance of gca%*	0.20	0.10	0.28	0.01	0.00
Relative importance of sca%**	0.79	0.89	0.71	0.98	0.99

shown in table 5. Additive and non-additive variances were significant for all studied traits. These results indicated that a major part of the total phenotypic variance for the studied characters was due to dominance genetic variances and doesn't affected by environmental effect indicating that dominance and over-dominance are preponderant in controlling the studied traits. Several workers also reported the predominance of dominant gene action for a majority of the yield traits. Satyanarayan *et al.*, 2000; Kumar *et al.*, 2004), while, Kumar *et al.* (1994) reported the predominance of additive gene and could be used in combination with other parameters action. Preponderance of non-additive gene action in the expression of yield and yield-related traits, was also reported by Pradhan *et al.* (2006) and Thirumeni *et al.* (2000). Heritability estimates in broad sense were high for the studied characters, whereas, heritability estimates in narrow sense heritability were relatively low for all studied characters. Therefore, it seems that hybridization must be a choice for utilizing the positive heterosis in special crosses. These results suggested that the materials under this study were very useful for explaining heterotic effect through hybrid rice for example, IR69625A × G46B, G46A × IR69625B, G46A × K17B, K17A × IR69625B and K17A × G46B. From these results could be concluded that the IR69625A / G46B, IR69625A / K17B, K17A / G46B and G46A / IR69625B considered as the best stable lines and using back cross method to develop new CMS lines.

The results in fig. 1 showed complete sterility for pollen grains which treated with potassium iodide for the three CMS lines; IR69625A/B, IR70368A/B and IR 58025A/B when marked crosses with their maintainers, the same results for pollen grains test were obtained for the cross, which marked among the IR69625A with K17B and G46B, also for the crosses among IR70368A with K17B and G46B, moreover the crosses among IR 58025A with K17B and G46B, these results were conformed with the data in table 1 for the up normal anther and pollen grains test ,that meaning could be develop new CMS line through back cross method among different sources of cytoplasmic male sterile.

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