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RESEARCH PAPER

Using line x 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 x 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 per cent seed set per cent and grain yield per plant⁻¹ there were ranged (86.33-100), (86.04-100), (71.57-87.04), (10.23-18.07) and (12.03-16.03) for up normal anther and color of pollen grains as well as, panicle exertion per cent and grain yield per plant⁻¹, whereas, testers were recorded 0.01 for anther shape and Wight colour of pollen grains and (97.00-99.67), (92.05-95.13) and (38.10-45.33) for panicle exertion per cent, seed set per cent and grain yield per plant⁻¹ 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 programme.

Key Words : Using line x tester, Develop new source, Cytoplasmic male sterile line, Hybrid rice

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Revealed to reach 8 billion by 2030 and rice production must be increased by 50 per cent 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 per cent higher yield just by growing hybrid rice instead of the common varieties (Virmani *et al.*, 1997) and in China gave more than 30 per cent yield advantage over conventional pure rice production by nearly 200 million tons from 1976 to 1991

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(Yuan, 1998). China is the first country to exploit commercially heterosis in rice. Hybrid rice technology was successfully developed in 1976 using male sterilityfertility 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, colour and panicle exsertion as well as shape and colour of pollen grains are related to cytoplasmic male sterile trait which using selection in early generation for sterile plant type. There for, 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.

Research Methodology

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 F₁ crosses were sown in the nursery on May 1st and seedlings were transplanted after 30 days. The parents and their F₁ 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 a (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 colour, panicle exsertion per cent, seed set per cent 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_b^2) and narrow sense heritability (h_n^2) were also estimated according to Falconar and Mackey (1996).

RESULTS AND REMONSTRATION

The results obtained from the present investigation as well as relevant discussion have been summarized under following heads :

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 x G46B and G46A x 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 x 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 colour of pollen grains, CMS lines IR69625A and G46A, the crosses of IR69625A x IR70368B, IR69625A x IR58025B, IR69625A x G46B, IR70368A x IR58025B, IR58025A x G46B and G46A x 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 x IR58025B), these result were confirmed with the data which recorded on

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Table 1 : The mean perform	Up normal	Wight colour of	Panicle exsertion	Seed set %	Grain yield per
Genotypes	anther (%)	pollen grain	(%)	unclosed panicle	plant ⁻¹
CMS line	-	-	-		-
IR69625A	100.00	100.00	87.04	18.07	16.03
IR70368A	93.31	93.34	82.63	12.10	14.07
IR 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 combination	-	-	-	-	-
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
R69625A *IR 58025B	100.00	100.00	73.87	11.37	14.00
R69625A *K17B	86.72	86.69	71.37	8.60	13.03
R69625A * G46B	100.00	100.00	57.37	15.37	16.00
R70368A IR69625B	95.10	92.33	90.87	11.10	15.05
R70368A *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

anther shape. Concerning to the panicle exsertion, the highest values for panicle exsertion were observed with CMS line IR 69625A (87.04%), tester IR69625B (99.67) and the cross G46A x IR 69625B (80.69%). On the other hand, the lowest values for panicle exsertion per cent were observed with CMS lines K17A (71.57%), tester IR58025B (97%) and the cross IR69625A x 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 per cent, the highest values for seed set per cent were recorded of CMS line IR69625A (18.07%), the tester (>90%) and the crosses IR 69625A x IR69625B and IR58025A x IR58025B, respectively, but, the lowest values for seed set per cent 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 x G46B gave desirable mean values (16.03, 45.33 and 18.12 g), respectively. While, the lowest values for grain yield were recorded of CMS line K17A, the tester K17B and the cross K17A x K17B (11.03, 38.10 and 11.03 g plant¹), respectively. The results of the general mean, for the lines, testers and F₁ crosses of the studied characters, indicated that, up normal anther, pollen grain colour, panicle exsertion per cent, seed set per cent 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 line x 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 epitasis) genetic variance were of great importance in the inheritance of these characters. These results revealed that dominance and dominance x 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 MS GCA/MS SCA 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

		Character						
S. O. V	d. f	Up normal Anther (%)	Wight colour of pollen grain	P. exsertion (%)	Seed set % unclosed panicle	Grain yield per plant ⁻¹		
Reps	2	0.02 NS	0.03 NS	1.05 NS	0.01 NS	0.01 NS		
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**		
L x 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 *** indicate significance of values at P=0.01, 0.05 and 0.1, respectively

NS= Non-significant

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Table 3 : Estimates of GCA et	Up normal anther	Wight colour of	Panicle exsertion	Seed	Grain yield
Genotypes	(%)	pollen grains	(%)	set (%)	per plant ⁻¹
CMS lines	-	-	-	-	-
IR69625A	6.1540	9.4792	-1.9760	1.6825	0.5320
IR70368A	2.4873	1.3532	11.1840	-1.2581	-0.2647
IR 58025A	4.7553	4.1745	-1.5493	1.9485	1.4287
K17A	-15.5473	-19.2461	-5.5560	-1.8241	-0.6280
G46A	2.1507	4.2392	-2.1027	-0.5488	-1.0680
(Testers)	-	-	-	-	-
IR69625B	2.0500	2.1292	5.9040	0.4159	0.3687
IR70368 B	-1.8453	1.5239	4.3173	-0.2888	0.3153
IR 58025B	3.4993	5.2412	-0.5693	-0.0448	-0.7680
K17B	-4.5187	-9.0981	-4.6493	-1.4115	-1.2580
G46B	0.8147	0.2039	-5.0027	1.3292	1.3420
L.S.D. for CMS lines 0.05	0.062	0.091	0.885	0.068	0.038
0.01	0.082	0.121	1.177	0.090	0.050
L.S.D. for testers 0.05	0.062	0.091	0.885	0.068	0.038
0.01	0.082	0.121	1.177	0.090	0.050

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	2.51600	5.13680	0.84467
IR69625A *IR70368 B	4.50	1.21	3.40267	-3.05853-	-0.10200
IR69625A *IR 58025B	-0.84	-2.51	1.52267	-1.10253	-0.05200
IR69625A *K17B	-6.10	-1.49	3.10267	-2.50253	-0.52867
IR69625A * G46B	1.84	2.53	-10.54400	1.52680	-0.16200
IR70368A IR69625B	-0.63	1.06	-1.11067	1.11080	0.65800
IR70368A *IR70368 B	1.48	2.67	0.27600	2.81547	-0.27200
IR70368A *IR 58025B	2.82	5.61	-1.97067	-2.42853	-0.25533
IR70368A *K17B	-2.50	6.59	-6.19067	-1.12853	-0.76533
IR70368A *G46B	-1.17	-15.94	8.99600	-0.36920	0.63467
IR 58025A* IR69625B	1.67	5.74	-1.51067	-6.12920	-3.08533
IR 58025A *IR70368 B	5.90	6.34	-2.39067	1.80880	1.31800
IR 58025A *IR 58025B	-6.03	-10.59	-2.20400	3.36480	0.08467
IR 58025A *K17B	-4.78	-9.32	4.27600	-0.26853	0.62467
IR 58025A *G46B	3.24	7.83	1.82933	1.22413	1.05800
K17A* IR69625B	-6.26	-6.35	2.46267	0.51347	1.17133
K17A *IR70368 B	-0.49	10.03	-0.05067	-1.48520	-0.97533
K17A *IR 58025B	0.89	11.34680	-0.39733	0.17080	0.17467
K17A *K17B	2.21	-12.84720	-1.11733	2.63747	-1.36867
K17A *G46B	3.64	-2.18253	-0.89733	-1.83653	0.99800
G46A* IR69625B	4.61	-0.71653	-2.35733	-0.63187	0.41133
G46A *IR70368 B	-11.39	-20.25453	-1.23733	-0.08053	0.03133
G46A *IR 58025B	3.16	-3.86187	3.04933	-0.00453	0.04800
G46A *K17B	11.18	17.06747	-0.07067	1.26213	2.03800
G46A *G46B	-7.55	7.76547	0.61600	-0.54520	-2.52867
L.S.D 0.05	0.138	0.204	1.979	0.151	0.084
0.01	0.184	0.271	2.632	0.201	0.112

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Table 5 : Estimates genetic parameters and heritability in broad and narrow senses for the studied characters during 2015 season						
Genetic parameters and heritability	Up normal anther (%)	Wight colour of pollen grains	Panicle exsertion (%)	Seed set (%) un closed panicle	Grain yield per plant ⁻¹ (g)	
Additive variance (o2A)	9.70337	13.95241	7.71672	0.09107	0.16116	
dominant variance (o2D)	36.81055	118.98617	19.33441	8.17391	22.85249	
Genotypic variance (σ 2G)	46.51392	132.9385	27.0511	8.2649	23.0135	
Environmental variance (σ 2E)	0.0295	0.0638	3.9833	0.0272	0.1063	
Phenotypic variance (σ 2P)	93.05734	265.9408	58.0855	16.5570	46.13345	
Broad sense heritability $(h_b^2\%)$	99.969	99.977	90.208	99.793	99.734	
Narrow sense heritability $(h^2_n\%)$	20.8548	10.4929	25.7333	1.0996	0.6984	
Relative importance of GCA%*	0.20861	0.10495	0.28526	0.01101	0.0070	
Relative importance of SCA%**	0.79138	0.895046	0.71473	0.9889	0.99300	



variation for these traits through hybrid breeding (Ramalingam *et al.*, 1997 and 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 per cent and wight colour of pollen grains, whereas IR58025A was recorded good combiner for seed set per cent and grain yield per plant⁻¹ and the line IR 70368 A was the best combiner for panicle exsertion per cent. Similar findings have been reported by Surek and Korkut (2002). The testers, IR58025B were the best general combiner for up normal per cent and wight colour of pollen grains whereas, the testers IR69625B were recorded best general combiner for panicle exsertion per cent, G46B was recorded best general combiner for seed set and grain yield per plant⁻¹ and could be used these parents in breeding programmes 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. The cross combinations G46A x K17B was the best specific combination for up normal anther and wight colour of pollen grains, the cross combination IR70368A x G46B were the best specific combiner for panicle exseration per cent. Concerning to seed set per cent the hybrid combination IR 69625A x IR 69625B was recorded the best specific combining ability for this characters, while, the hybrid cross G46A x 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 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. Satyanarayana et al., 2000 and Kumar et al., 2004), while Kumar et al. (1994) reported the predominance of additive gene and coud 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 x G46B, G46A x IR69625B, G46A x K17B, K17A x IR69625B and K17A x 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.

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