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IDENTIFICATION OF SOME WIDELY COMPATIBLE RESTORER LINES USING TESTCROSS AND SSR MARKERS

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ABSTRACT

Hybrids between distantly related high yielding varieties are most promising. Discovery and exploitation of widely compatible varieties provide a possibility for the utilization of heterosis between Indica and Japonica types. Investigation was carried out at the farm of Rice Research &Training Center (RRTC), Sakha Kafr El-Sheikh, Egypt from 2008 at 2010. To evaluate the presence of restorer and wide compatibility genes for 85 lines of F_5 developed from the cross between Giza178 and Dular. Six SSR markers were used to detect the presence of restoring ability (Rf) and wide compatibility (WC) genes. A total of 19 lines selected by SSR markers were crossed as male (testers) with two Indica cytoplasmic male sterile lines (IR58025A and IR69625A) and two Japonica CVS; Sakha101 and Giza177 as female parents to produce 76 F_1 hybrids seeds according to 4 x19 Line x Tester fashion. Results of SSR markers indicated that Dular variety had two alleles (S-5 and S-8) for wide compatibility (WC) While, Giza178 had two Rf alleles; Rf-3 and Rf-4. However, the selected lines from F_5 generations holded at least one Rf allele either Rf3 or Rf4 and one WC allele either S-5 or S-8. Some lines had the four alleles, two alleles (Rf3 and Rf4) for restoring ability, and two alleles (S-5 and S-8) for wide compatibility. All 19 testers showed high pollen and spikelets fertility% (more than 80%) with Japonica testers, Sakha101 and Giza177 while, 13 lines showed pollen and spikelets fertility more than 80%. with Indica CMS lines (IR58025A and IR69625A). These results indicated that WC alleles and restoring ability alleles (Rf) are present in these lines and these lines can be used to produce Indica/ Japonica hybrids. For heterosis of grain yield plant^{-1}, most of crosses exhibited significant heterosis with positive values ranged between 17.31 and 59.34 %.

Keywords: Heterosis, rice, hybrid, wide compatibility, restoring ability and CMS.

INTRODUCTION

Exploitation of heterosis has played a significant role to increase productivity of several crops including rice. Availability of suitable pollination control systems and the extent of out crossing between female and male parents are the key factors determining the success of commercial exploitation of heterosis in any crop.Cytoplasmic-genetic male sterility (CMS) combined with a fertility restoration system has been found to be the most efficient genetic tool to exploit hybrid vigor on a commercial scale in rice (Lin and Yuan 1980 and Virmani and Shinjyo 1988). The wild abortive cytoplasmic male sterility (CMS-WA) system, an ideal type of sporophytic CMS in Indica rice, is used for the large scale commercial production of hybrid rice. In hybrid rice breeding program the extent of heterosis depends on the relation between parental lines. Hybrids between distantly related high yielding varieties are most promising. Indica and Japonica rices are genetically

diverse therefore; Indica/Japonica hybrids are showing strong heterosis for various traits, including total dry matter, number of tillers, number of spikelets and 1000-grain weight. However, crosses between Indica and Japonica varieties generally showed low fertility in F_1 and segregated a wide range of semi-sterile plants in F_2 as well as subsequent generation. These conditions limited the recovery of certain recombinants. If there were any means to solve this problem, rice breeding involving Indica/Japonica crosses would become more efficient (Kumar and Virmani, 1992 and Julfiquar, 1993). On the other hand, some varieties could produce fertile F_1 hybrids when crossed with Indica or Japonica lines, these varieties were designated as wide compatibility varieties (WCVs). The discovery and exploitation of widely compatible varieties provided a possibility for the utilization of heterosis between Indica and Japonica.The key to this approach is to introduce widely compatible genes into the CMS lines for developing the widely compatible CMS lines. Additional strategies need to be deployed to develop widely compatible restorer (WCR) lines which show strong heterosis and good restoration ability, and compatibility to both Indica CMS lines (WA cytoplasm) and Japonica CMS lines (BT cytoplasm). Recently the advances in molecular marker technology have enabled several research groups to determine the chromosomal locations of the two Rf genes (Rf3 and Rf4) for the WA-CMS system. This investigation aims to, i) Incorporate wide compatibility gene into restorer lines. ii) Detect the widely compatible restorer lines by SSR markers. iii) Confirm the results of SSR markers by testcross and checking of pollen and spikelets fertility.

MATERIALS AND METHODS

Investigation was carried out at Rice Research &Training Center (RRTC) Farm, Sakha Kafr El-Sheikh, Egypt from 2008 to 2010. To evaluate the presence of restorer and wide compatibility genes in 85 lines F_5 developed from the cross between Giza178 and Dular. Dular was used as donor for wide compatibility gene while, Giza 178 was used as donor for restorer genes as reported by Kumar and Virmani, (1992), and Bastawisi *et al.*, (2002). Six SSR markers were used to detect the presence of restoring ability (Rf) and wide compatibility (WC) genes Table 1. Four SSR markers linked to Rf genes, RM315 and RM443 have been reported as flanking markers to (Rf3) (Bazarkar *et al.*, 2008), while RM171 and RM258 was flanking to (Rf4), (Nematzadeh and Kiani, 2010). At the same time two markers namely RM253 and RM412, have been reported as markers linked to S-5 and S-8 WC genes, respectively. In addition linkage of morphological markers Chromogenic of apiculus color with WC gene, (Singh *et. al.*, 2006 and El-Namaky, 2008). In 2008 85 lines F_5 screened by SSR markers for WC and Rf19 alleles, in 2009, 19 promising lines which showed linkage with both Rf and WC were crossed as male parents (testers) with two Indica cytoplasmic male sterile lines (IR58025A and IR69625A) and two Japonica lines (Sakha101 and Giza177) as female parents (lines) to produce 76 F_1 hybrids seeds according to (4 x19) Line x Tester fashion. In 2010 all the

genotypes were evaluated in a Randomized Complete Blocks Design (RCBD) experiment with three replications. The observations were recorded on ten plants which were taken at random from each test entry in each replication to detect for, i.e. Pollen fertility (%), spikelets fertility (%), filled grains panicle^{-1}, plant height, days to50% flowering, and grain yield plant^{-1}, according to IRRI SES, 1996. Fertility and sterility classification was recorded according to Virmani 1998. Pollen fertility and seed-setting rate were used as the main criteria for the evaluation of fertile and sterile plants. Mature anthers were harvested, and the pollen was stained with 1 % I2-KI solution. The numbers of dark blue (stainable) and clear pollen grains (un-stainable) in each individual were counted under an optical microscope, and the numbers of the seed set on a spikelet were counted. Fertility evaluation was conducted as described by Li *et al.*, (2005).

DNA isolation, purification and quantification: DNA isolation and purification were carried out using CTAB (Cetyl-tetramethyl ammonium bromide) method, (Murray and Thompson, 1980). DNA Polymorphism was carried out in PCR programmed using SSR primers. The simple sequence repeats primer pairs (SSR) namely (RM315, RM443) and (RM171, RM258) have known to be linked to restoring ability alleles, Rf3, and Rf4, respectively to be used for validation. Two specific primers RM253 and RM412 known to be linked to wide compatibility genes S-5 and S-8, respectively, (Table 1). The primers pairs introduced from Ferments Company Germany and it sequences were directly downloaded from gramene website (www.gramene.org). PCR reaction volume was 10 µl PCR volume containing 50 ng template DNA, 5 mole (13 ng) of each of forward and reverse primers, 0.1 mM dNTP's, 1x PCR buffer (10 mM Tris, pH 8.0, 50 mM KCl and 50 mM ammonium sulphate), 1.8 mM MgCl2, and 0.2 unit Taq polymerase (Ferments). The PCR cycling conditions involved initial denaturation at 94°C for 5 minutes followed by 35 cycles at 94°C for 1 min, primer annealing at 55.7°C for 1 min. and primer extension at 72°C for 2 min. By the end of 35th cycle, final extension at 72°C for 5 minutes was given, followed by storage at 4°C. PCR thermocycler machine from Biometra and Applied Bio systems was used.

Table 1: Bases sequences of SSR primers used with rice.

No.	Allele	Marker ID	Ch	Forward Sequence	Reverse Sequence
1	RF3	RM315	1	CGGTCAAATCATCACCTGAC	CAAGGCTTGCAAGGGAAG
2	RF3	RM443	1	GATGGTTTTCATCGGCTACG	AGTCCCAGAATGTCGTTTCG
3	RF4	RM171	10	AACGCGAGGACACGTACTTAC	ACGAGATACGTACGCCTTTG
4	RF4	RM258	10	TGCTGTATGTAGCTCGCACC	TGGCCTTTAAAGCTGTCGC
5	WCG S-5	RM253	6	TCCTTCAAGAGTGCAAAACC	GCATTGTCATGTCGAAGCC
6	WCG S-8	RM412	6	CACTTGAGAAAGTTAGTGCAGC	CCCAAACACACCCAAATAC

RESULTS AND DISCUSSION

Detection of restoring ability (Rf) and wide compatibility genes by SSR markers: 85 lines screened by six SSR primers to detect the presence of

restoring ability (Rf) and wide compatibility (WC) genes. Results of 19 selected lines by SSR markers and their parents Dular and Giza178 are presented in the figures, 1 to 6.

Markers linked to restoring ability:

Rf3: is a major restorer gene to CMS wild abortive type (WA), flanked by two co-dominant SSR markers, RM315 and RM443, chromosome 1 (Majid *et al.*, 2007). The control rice restorer in this study is Giza178 so any line that produce similar PCR product to Giza178 will be considered to have the Rf3. Results of 19 lines of F_5 and both parents (Giza178 and Dular) screened by RM315 maker are presented in Fig.1. The results showed that Giza178 and 15 Lines F_5 lines have Rf allele A, while Dular and other lines have allele B. Data in Fig. 2, showed that, amplification of RM443, for 19 F_5 lines and their both parents. The results showed that, 12 promising lines have Rf allele A, (similar allele located in Giza178), while Dular and other lines have allele B. According to the results of the two flanking markers, nine lines was holding Rf3 gene. So, these promising lines could be used as restorer lines for CMS lines (WA) (Shaoqing, *et al.*, 2005). This finding proofs the success of selection among F_5 generation lines to produce lines with Rf3 allele.

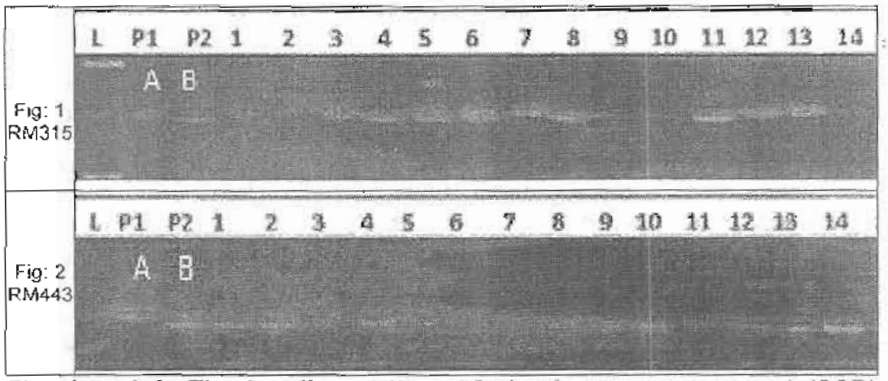

Fig. 1 and 2: The banding pattern of simple sequence repeat (SSR) markers RM315 and RM443 (linked with Rf3) for the two parents and selected lines, (1-19). P1= Giza178, P2= Dular 1-19= WCR line.

Rf4: is a restorer gene flanked by RM171 and RM258 SSR markers on chromosome 10, the genetic distances 2.1 cM and 2.9 cM, respectively according to Ahmadikhah and Karlov, (2006) and Shaoqing *et al.*, (2007). The RM171 marker banding pattern showed that 10 lines holded allele A (same allele located in Giza178), while Dular and other lines showed allele B, Fig.3. On the other hand, their banding pattern using RM253 marker revealed that 7 lines have the same Giza178 allele (allele A), Fig 4. The results of the two Rf4 flanking markers indicated that 12 lines have Rf allele linked with RM171 or RM253. These results indicated that these 12 promising lines have Rf4 allele, these lines could be used as restorer for CMS lines WA or HL types, Shaoqing *et al.*, (2005).

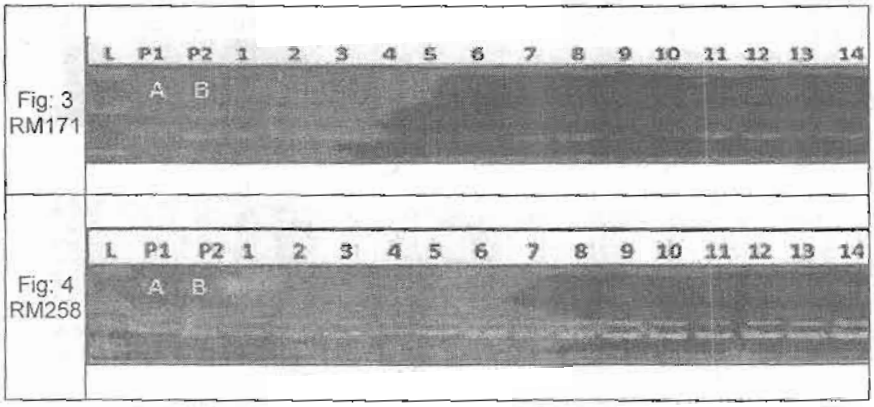

Fig. 3 and 4: The banding pattern of simple sequence repeat (SSR) markers RM171 and RM258 (linked with Rf4) for the two parents and selected lines, (1-19). P_1= Giza178, P_2= Dular 1-19= WCR line.

Marker linked to WC genes:

Some rice varieties could produce fertile F_1 hybrids when crossed with Indica or Japonica lines, these varieties were designated as wide compatibility varieties (WCVs). There are two QTLS controlling wide compatibility traits, S-5 major allele and S-8 minor allele on chromosome 6. RM253: is a co-dominant marker that linked to wide compatibility gene S-5, on chromosome 6 (Singh *et. al.*, 2006). Dular variety is known to be a wide compatibility variety (WCV) Kumar and Virmani, (1992) , so any line that produce similar PCR product to Dular will be considered to have S-5 allele. The results in Fig.5 showed that Dular and 13 promising lines holded the S-5 allele A while, Giza178 and other lines holded allele B. On the other hand, RM412 primer is a co-dominant marker that tightly linked to a minor gene for wide compatibility (S-8 locus) on chromosome 6 (Wan *et al.*, 1993). The results in Fig.6 showed that nine promising lines have the Dular allele A. While Giza178 and other selected lines involved S-5 allele B. The results of the two Rf4 flanking markers indicated that these promising lines have WC gene and it could be used to overcome sterility in Indica / Japonica crosses, according to (Singh *et al.*, 2006 and Qing *et al.*, 2010).

In general, the selected lines from F_5 generation holded at least one Rf allele (Rf3 or Rf4) and one WC allele (S-5 or S-8). Some lines had the four alleles, two alleles (Rf3 and Rf4) for restoring ability, and two alleles (S-5 and S-8) for wide compatibility. In the same time 11 lines (1, 3, 4, 5, 6, 8, 10, 11, 14, 16 and 19) include at least the two major alleles (Rf3) for restoring ability and S-5 for wide compatibility. These promising lines might have WC and Rf genes. Thus, it could be used as widely compatible restorer lines with Indica and Japonica CMS lines to produce Indica / Japonica hybrid.

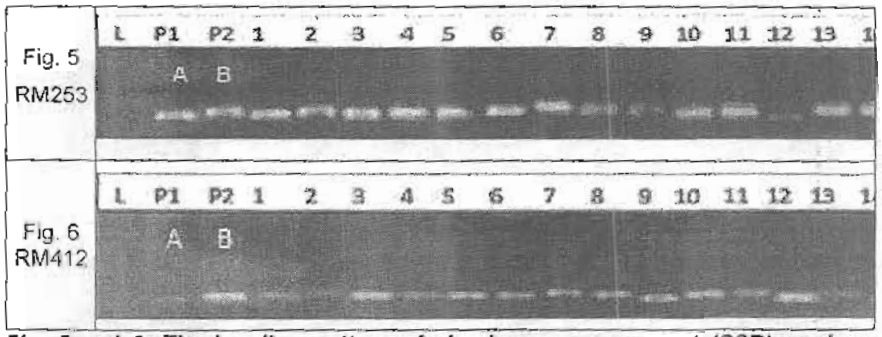

Fig. 5 and 6: The banding pattern of simple sequence repeat (SSR) markers RM253 and RM412 linked with WC genes (S-5 and S-8, respectively) for the two parents and selected lines, (1-19).

P_1= Giza178
P_2= Dular 1-19= WCR line.

Testcross:

Based on molecular screening 19 promising lines were crossed with two Indica CMS lines (IR58025A and IR69625A) and two Japonica lines (Sakha101 and Giza177) to confirm the presence of wide compatibility and restoring ability in these lines. Pollen and spikelet fertility % were the main criteria to confirm the results, in addition some agronomic traits were recorded.

Table 2: Mean square estimates from line x tester analysis of variance for all studied traits.

S.V	d. f	Days to 50% flowering	plant height (cm)	No. of field grains panicle	pollen fertility%	Spikelets fertility%	Grain yield /plant
Treatments	98	30.2**	62.11**	2169.5**	640.5**	610.6**	1086.9**
Parents	22	68.9**	78.49**	4503.6**	2142.7**	2060.0**	2885.5**
Crosses	75	18.7**	48.70**	795.7**	207.7**	187.8**	456.4**
Par. vs. crosses	1	45.7**	707.34**	53851.8**	55.5**	430.1**	8816.6**
Lines	18	44.8**	98.67**	1694.8**	333.1**	255.3**	887.6**
Testers	3	10.0**	143.61**	667.76**	913.7**	994.4**	885.5**
Lines x testers	54	10.5**	26.77**	503.1**	126.7**	120.5**	288.0**
Error	196	1.42	10.85	40.8	10.5	11.5	14.8

**= Highly significant

Analysis of variance:

Analysis of variance of Genotypes, Parents, Crosses, Par.Vs.Cross, Lines, Testers and lines x Tester for all studied traits .i.e. days to50% flowering, plant height (cm), filled grains panicle^{-1}, pollen fertility (%), spikelets fertility (%), and grain yield plant^{-1} are presented in Table 2. The obtained results indicated that the mean squares of Genotypes, Parents, Crosses, Par. vs. Cross, Lines, Testers and Lines x Tester were highly significant with respect to all the studied traits. This finding indicated the presence of large

variations among the studied genotypes and the partition of these genotypic variances to its components are varied. Similar results were previously obtained by El-Namaky (2003), Ahmed (2004), and El-Mowafi (2006).

Mean performance:

Lines and testers: Mean performances of lines and tester for all studied traits are presented in Table 3. Most of the tester lines were early flowering ranged from 81.3 to 91.3 days compared with the four lines which ranged between 95.7 and 100.3 days. For plant height both lines and testers varied from short and medium stature ranged between 88.7 to 105.0 cm. Concerning, number of filled grains panicle^{-1} Sakha101 showed the highest number of filled grains panicle^{-1} (152) among lines and testers. Regarding% pollen and %spikelet fertility all lines and testers showed fertility percentage higher than 88%, except both (CMS) lines IR58025A and IR69625A were complete sterile lines. For grain yield plant^{-1}, WCR18 had the highest mean values for grain yield plant^{-1} (71.67 g) among lines and testers.

F_1.Testcross: Performances of 19 testers with two Indica CMS lines (IR58025A and IR69625A) and two Japonica lines (Sakha101 and Giza177) are presented in Table 4. The results indicated that generally most of the 19 testers showed high pollen and spikelets fertility% (more than 80%) with Japonica lines, (Sakha101 and Giza177) and Indica lines (IR58025A and IR69625A). This indicated the presence of wide compatibility alleles (WC) in these testers and these breeding lines can be easily crossed with Indica and Japonica varieties without sterility. All 19 lines exhibited high pollen and spikelets fertility with Japonica lines, (Sakha101 and Giza177)) these indicating that the presence of WC alleles in these lines. Out of the19 testers crossed with Indica lines (IR58025A and IR69625A), 13 lines showed pollen and spikelets fertility more than 80%.This finding illustrated that WC alleles and restoring ability alleles (Rf) are present in these lines which can be used to produce Indica/ Japonica hybrids. Xie *et al.*, (1997) reported that the wide compatibility (WC) was incorporated into restorer lines obtained; their value in intersubspecific hybrid rice breeding was high yield potential in the field by crossing Japonica WC lines with Indica lines. Yang (1997) selected several widely compatible R lines from anther culture of F_1 hybrids for Indica R line/WCVs and Japonica R line/WCVs. These widely compatible Restorer lines derived from diploid pollen and they showed a good widely compatible and restoring ability both to WA and BT type CMS lines. While, four testers (WCR2, WCR7, WCR15 and WCR19) showed pollen and spikelets fertility % ranged between 60.1 and 77.1% with Indica CMS lines. These results indicated that the absences of Rf alleles in these lines or maybe some inhibitor genes linked with Rf genes. These results in the same trend with other studies which indicated that a different levels of fertility for Rf due to interaction between Rf alleles and another alleles in some female parents. These results cleared when the same restorer lines give different level of fertility with different CMS lines WA. In the same time two testers, WCR16 and WCR17 revealed that high pollen fertility% with both Indica CMS lines while; showed spikelets fertility %, 69.5 and 71.2% with CMS lines IR58025A and 91.2 and 83.2% with CMS line IR69625A. These results demonstrated

that pollen and spikelets fertility% were independent traits. These results agree with, those obtained by Vijaya Kumar (1988) who reported three types of pollen and spikelets fertility% viz. low pollen and high spikelets fertility%, high pollen and low spikelets fertility% and normal pollen and spikelets fertility% of different relationship between pollen and spikelets fertility% of the F_1 hybrids of certain cultivars. Grain yield/plant: Large variations of grain yield/ plant were noticed in F_1 hybrids which ranged between 22 and 74.3g. Nine crosses (IR58025A/WCR6, IR58025A/WCR8, IR69625A/WCR1, IR69625A/WCR8, Giza177/WCR3, Giza177/WCR9, Sakha101/WCR1, Sakha101/WCR4 and Sakha101/WCR9) exhibited grain yield ranged from 70 to 74.3g plant^{-1}. Generally, most of the hybrids revealed grain yield plant^{-1}, higher than parents this indicated the presence of hybrid vigor and over in these crosses. Concerning days to 50% flowering, most of the crosses were early maturity (Table 5). The days to 50% flowering ranged between 82.2 and 92.2 days. These results revealed that there was no variation for days to flowering among the cross and the effect of day length and temperature degree on day to flowering. Regarding plant height the crosses varied from semi-dwarf (92.3 cm) to medium tall (112 cm), the crosses Sakha101/WCR15, IR69625A/WCR14 and Giza177/WCR13 were the shortest stature. Large variations of number of filled grains panicle^{-1} were observed and the mean values ranged between 96.4 and 172.4 grains panicle^{-1}.

Heterosis:

Rice variety Sakha101 had the highest grain yield in Egypt and subsequently cultivated in large area. It was used as check variety to estimate the standard heterosis of grain yield plant^{-1} for the 76 F_1 hybrids. The estimates of heterotic values related to standard check for grain yield plant^{-1} of 76 F_1 hybrids developed from crosses between four lines and their 19 testers are presented in Table 6. The results demonstrated that 11 testers (WCR) exhibited significant heterosis with positive values with both Indica lines, IR58025A and IR69625A. While the tester WCR12 exhibited significant heterosis with positive values with IR69625A only. The crosses IR58025A/WCR8 and IR69625A/WCR8, showed the highest and significant positive estimates, 55.91 and 57.19%, respectively. While four testers, WCR2, WCR7, WCR15 and WCR19 revealed significant and negative heterosis with Indica CMS lines. In the same time 16 testers gave significant heterosis and positive values with Japonica line Giza177, and 13 testers exhibited significant heterosis with positive values with Japonica line Sakha101. The heterosis with Japonica lines Sakha101 and Giza177 ranged between 17.31 and 59%. Also, the heterosis with Indica lines (IR58025A and IR69625A) ranged between 17.74 and 57.19%. These results indicate that high heterosis with positive values was obtained for most of F_1 hybrids and the WCR lines can be used as restorer with Indica and Japonica CMS.

Table 3: Mean performances for 19 lines and their four testers for all studied traits.

Genotypes	Days to 50% flowering	Plant height (cm)	No. of filled grains panicle^{-1}	Pollen fertility%	Spikelets fertility%	Grain yield plant^{-1} (g)
R58025A	100.3	101.3	0.01	0.01	0.01	0.01
R69625A	97.0	105.0	0.01	0.01	0.01	0.01
Sakha101	99.0	100.8	152.0	95.40	92.57	46.63
Giza177	95.7	104.0	144.5	95.13	92.67	43.40
WCR1	87.0	100.8	137.5	93.50	94.07	48.00
WCR2	88.3	102.3	139.6	93.77	91.80	48.00
WCR3	81.3	88.7	123.7	90.62	89.13	53.00
WCR4	83.7	95.7	130.0	88.11	87.65	41.67
WCR5	89.3	89.8	128.6	89.77	91.35	45.67
WCR6	90.7	96.5	137.1	91.22	89.76	32.67
WCR7	89.0	93.2	127.3	94.96	91.20	49.33
WCR8	90.0	99.2	130.8	94.23	92.86	43.67
WCR9	84.3	98.5	122.8	92.48	91.68	40.00
WCR10	85.3	104.3	119.7	93.87	90.63	42.00
WCR11	86.3	104.0	128.0	92.49	92.00	36.67
WCR12	87.7	95.3	125.8	93.51	91.03	30.67
WCR13	87.0	89.3	115.6	92.60	87.63	32.00
WCR14	90.0	101.0	130.0	89.61	88.47	34.67
WCR15	86.3	104.0	126.9	90.15	91.36	35.00
WCR16	91.0	100.5	132.7	93.92	90.28	46.67
WCR17	86.7	94.7	136.1	92.30	90.47	48.00
WCR18	87.0	104.5	141.1	94.28	89.41	71.67
WCR19	91.3	93.8	131.1	90.69	90.31	33.00
LSD 0.05	1.94	5.35	10.38	5.20	15.07	14.14
0.01	2.59	7.16	13.89	6.79	19.69	18.31

Table 4: Percentages of pollen, spikelets fertility and grain yield plant^{-1} of 19 testers with four lines.

Testers	Pollen fertility%				Spikelets fertility%				Grain yield plant^{-1} (g)			
	Japonica Lines		Indica Lines		Japonica Lines		Indica Lines		Japonica Lines		Indica Lines	
	B1	B2	A1	A2	B1	B2	A1	A2	B1	B2	A1	A2
WCR1	84.4	88.7	91.4	88.4	86.5	91.4	89.6	89.6	54.9	70	61.3	72.7
WCR2	63.4	63.7	90.4	88.1	62	62.3	89.8	94.3	26	22	62.7	68
WCR3	86.8	91.1	86.6	90	87.3	89.3	91.1	88.5	64.3	62.3	72.7	64.3
WCR4	85.3	90	90.1	88.7	85.5	88.9	87.3	93.5	63.0	64.0	65.7	72.0
WCR5	87.7	87.4	88.8	90.1	89.6	89.6	88.1	92.3	65.7	61.3	62.0	64.0
WCR6	84.4	92	89.9	89.5	88.5	88.8	89.8	90.5	71.0	69.0	60.3	62.7
WCR7	63.3	60.9	87.8	92.6	63.5	77.1	88	91.5	27.3	28.0	58.3	63.7
WCR8	84.6	91.4	86.5	89.8	86.9	90.3	88.7	89.3	72.7	73.3	53.3	62.0
WCR9	85.4	90.2	86.8	90	86.5	89.8	90.3	87.2	62.7	62.5	71.3	74.3
WCR10	87.2	87.3	92	86.9	88.8	93.5	88.9	86.5	50.9	51.3	48.7	52.7
WCR11	88.1	86.1	89.1	85.5	87.4	87.4	89	85.6	63.0	59.0	50.0	56.7
WCR12	90.2	85	92.6	87.3	88	88.3	87.8	87	50.3	60.7	49.3	63.0
WCR13	85.8	84.7	89.5	85.8	86.3	88.6	86.1	87.8	47.0	49.4	57.7	50.3
WCR14	88.6	86.9	90.5	88.4	84.9	88.2	89.6	89.5	45.0	41.3	55.3	56.0
WCR15	62.7	60.1	89.1	87.1	62.3	69	89.1	89.3	34.0	29.7	58.0	61.3
WCR16	85.8	88.7	91.5	84	71.2	91.2	90.1	86.1	58.6	63.0	59.7	56.3
WCR17	88.9	86.3	90.9	86.5	69.5	83.1	90.7	87.4	56.7	55.7	49.3	48.7
WCR18	86.5	85.9	91.1	87.2	89.6	79.6	90.7	88.6	63.7	61.3	40.3	57.3
WCR19	60.2	63.3	88.4	88.9	62.5	69.1	86.3	87.8	30.7	32.3	54.7	57.0
LSD 0.05	5.2				15.07				14.14			
0.01	6.79				19.69				18.31			

A1=IR58025A A2= IR69525A B1= Giza177 B2= Sakha101

Table 5: Days to 50% flowering, plant height and filled grains panicle^{-1} of 19 testers with four lines.

Testers	Days to 50% flowering(day)				Plant height (cm)				No. of filled grains panicle^{-1}			
	Japonica Lines		Indica Lines		Japonica Lines		Indica Lines		Japonica Lines		Indica Lines	
	B1	B2	A1	A2	B1	B2	A1	A2	B1	B2	A1	A2
WCR1	90.7	91.4	88.2	90.0	105.0	107.0	102.0	103.3	169.6	169.6	161.4	166.9
WCR2	90.8	92.2	88.2	85.3	106.0	102.0	101.8	100.3	104.8	107.7	153.2	158.3
WCR3	85.2	88.5	88.8	91.7	102.7	105.0	101.0	103.7	170.9	170.9	166.4	157.8
WCR4	85.8	85.3	89.5	84.3	106.7	111.3	107.0	103.3	150.9	162.5	151.2	155.4
WCR5	90.3	89.7	89.7	90.0	101.3	104.7	102.0	99.8	172.4	169.2	166.8	160.4
WCR6	89.5	90.8	90.8	90.3	104.7	111.0	102.8	101.2	167.7	172.1	157.1	160.3
WCR7	88.2	90.7	90.5	92.0	102.8	102.7	105.3	103.5	96.4	124.4	152.9	165.6
WCR8	86.3	91.0	92.0	89.3	112.0	105.0	101.3	102.7	150.1	156.9	150.1	161.2
WCR9	90.7	90.5	89.7	90.3	104.0	106.7	105.7	107.0	168.9	164.4	155.2	152.8
WCR10	92.2	91.2	90.0	93.0	110.3	102.7	105.0	102.0	168.5	172.9	165.1	160.7
WCR11	87.0	88.2	83.8	86.7	105.2	103.0	98.0	95.7	156.3	132.4	149.8	158.9
WCR12	88.5	89.2	83.5	82.7	101.5	105.0	104.5	100.8	152.6	159.7	145.3	152.9
WCR13	88.0	85.8	85.8	88.7	96.5	98.7	93.2	95.5	157.6	146.2	146.1	156.1
WCR14	82.8	82.2	86.2	87.7	102.8	92.7	94.8	101.7	166.5	136.1	149.0	149.4
WCR15	87.2	86.7	86.3	88.0	105.2	102.3	97.0	92.3	120.6	135.6	166.9	152.1
WCR16	86.5	87.8	82.3	87.3	97.5	95.7	103.2	101.5	154.4	136.7	147.2	141.9
WCR17	87.3	87.7	87.5	89.0	101.2	103.7	97.5	99.2	151.1	140.7	145.8	148.1
WCR18	85.8	87.7	88.3	88.0	107.7	104.7	98.7	102.7	145.6	150.1	154.7	154.4
WCR19	88.3	88.2	88.3	88.3	104	100.7	99.8	99.7	109.8	118.5	149.5	138.1
LSD 0.05	1.94				5.35				10.38			
0.01	2.59				7.16				13.89			

A1=IR58025A A2= IR69525A B1= Giza177 B2= Sakha101

Table 6: Heterosis estimates of grain yield plant^{-1} for 76 F_1 hybrids (4 Lines x 19 Testers).

Testers	Indica lines		Japonica lines	
	IR58025A	IR69625A	Giza177	Sakha101
WCR1	17.74*	50.12**	31.46**	55.91**
WCR2	-44.24**	-52.82**	34.46**	45.83**
WCR3	37.89**	33.60**	55.91**	37.89**
WCR4	35.11**	37.25**	40.90**	54.41**
WCR5	40.90**	31.46**	32.96**	37.25**
WCR6	52.26**	47.97**	29.32**	34.46**
WCR7	-41.45**	-39.95**	25.03**	36.61**
WCR8	55.91**	57.19**	14.30	32.96**
WCR9	34.46**	34.03**	52.91**	59.34**
WCR10	9.16	10.02	4.44	13.02
WCR11	35.11**	26.53**	7.23	21.60**
WCR12	7.87	30.17**	5.73	35.11**
WCR13	0.79	5.94	23.74**	7.87
WCR14	-3.50	-11.43	18.59**	20.09**
WCR15	-27.09**	-36.31**	24.38**	31.46**
WCR16	25.67**	35.11**	28.03**	20.74**
WCR17	21.60**	19.45**	5.73	4.44
WCR18	36.61**	31.46**	-13.57	22.88**
WCR19	-34.16**	-30.73**	17.31*	22.24**
LSD 0.05	15.74	15.74	15.74	15.74
0.01	17.75	17.75	17.75	17.75

*= Significant **= Highly significant.

In this respect, many authors obtained the same results among them Ahmed (2004) and El-Namaky (2008). Same trend, Hammoud (2004) reported that twenty six hybrid combinations were found to exhibit highly significant positive heterosis for grain yield plant^{-1} which ranged from 0.78 to 203.36% in the F_1 generation over the better parent for two years.

REFERENCES

Ahmadikhah, A. and G. I. Karlov (2006). Molecular mapping of the fertility-restoration gene Rf4 for WA-cytoplasmic male sterility in rice. Plant Breeding :125, 363—367.

Ahmed, A. R. M. (2004). Genetical studies on some hybrids of rice. . M.Sc. Thesis, Fac.of Agric Mansoura University, Egypt.

Bastawisi, A.O.; H.F. El-Mowafy; M.I. Abo-Youssef; A.E. Draz; I.R. Aidy; M.A. Maximos and A.T. Badawi (2002). Hybrid rice in Egypt.4th International Symposium on Hybrid Rice, 14-17, May 2002, Hanoi, Vietnam. 96: 526-538.

Bazarkar, L.; A.J. Ali; N.A. Babaeian; A.A. Ebadi; M. Allahghollipour; K. Kazemitabar; and Nematzadeh, G. (2008). Tagging four fertility restorer loci for wild abortive-cytoplasmic male sterility system in rice (*Oryza sativa* L.) using microsatellite markers. Euphytica 164 (3) pp. 669-677.

El –Namaky; R.A. (2003). Genetic studies on wide compatibility in rice (*Oryza sativa* L.). M.Sc. Thesis, Fac.of Agric Tanta University, Kafr El-Sheikh,Egypt.

El-Mowafi; H.F. (2006). Genetic and combining ability analysis of grain quality characters in hybrid rice. J .Agric. Sci . Mansoura University., 31(8): 4929-4945.

El-Namaky, R.A. (2008). Genetic studies on using of wide compatibility gene (WC) in hybrid rice breeding. Ph.D. Thesis, Fac. of Agric., Mansoura University, Egypt.

Hammoud, S.A.A. (2004). Inheritance of some quantitative traits in rice (*Oryza sativ,* L.). Ph.D. Thesis, Fac. of Agric., Minufiya University, Egypt.

IRRI, INGER. (1996). Standard Evaluation System for rice 4th edition.

Julfiquar, A.W. (1993). Identification of wide compatibility genes in rice (*Oryza sativa* L.).Ph.D. Thesis, Central Luzon State Univ., Munoz, Nueva, Ecjia, Philippines.

Kumar, V.R. and S.S. Virmani (1992).Wide compatibility in rice (*Oryza sativa* L.). In Euphytica (63): 71-80.

Li S; Q.; G.H. Yang; S.B. Li; Y.G. Zhu and Y.S. Li (2010). Distribution and inheritance of the fertility restorer genes for WA-CMS in wild rice with AA genome. *Acta Agron Sin*, 31(3): 297–301.

Lin S.C. and L.P. Yuan (1980). Hybrid rice breeding in China. In: IRRI (eds) Innovative approaches to rice breeding. International Rice Research Institute, Manila, Philippines, pp 35–51.

Majid, S.; A Kathiresan.; G. B. Gregorio; J. E. Hernandez; M. N. Tamerlane and S. S. Virmani (2007). Development and use of a two-gene marker-aided selection system for fertility restorer genes in rice. Euphytica 153:35–42.

Murray, M.G. and W.F. Thompson (1980). Rapid isolation of high molecular weight plant DNA. Nucleic Acids Res., 8:4321–4325.

Nematzadeh GA, A.J. Ali; M. Sattari; A. Valizadeh; E. Alinejad and M.Z. Nouri 2006). Relationship between different allogamic associated trait characteristics of the five newly developed cytoplasmic male sterile (CMS) lines in rice. J. Cent. Eur. Agric. 7: 49-56.

Nematzadeh. A. Gh and G. Kiani (2010). Genetic analysis of fertility restoration genes for WA type cytoplasmic male sterility in Iranian restorer rice line DN-33-18. African Journal of Biotechnology 9(38): 6273-6277.

Qing Ji ,Jufei Lu , Qing Chao, Yan Zhang, Meijing Zhang, Minghong Gu and Xu Mingliang (2010). Two sequence alterations, a 136 bpInDel and an A/C polymorphic site, in the S5 locus are associated with spikelet fertility of indica-japonica hybrid in rice, J. Genet. Genomics 37: 57-68

Shaoqing Li, Daichang Yang and Yingguo Zhu (2007). Characterization and use of male sterility in hybrid rice breeding, Journal of Integrative Plant Biology, 49 (6): 791−804

Shaoqing Li; G.Yang; S. Li; Y. S. Li; Z. Chen and Y. Zhu (2005). Distribution of fertility-restorer genes for wild-abortive and honglian CMS lines of rice in the AA genome species of genus *Oryza*. Annals of Botanty, 96:461-466.

Singh S.P.; R. .M..Sundaram; S.K. Biradar; M. I. Ahmed; B. C. Viraktamath and E.A. Siddiq (2006). Identification of simple sequence repeat markers for utilizing wide compatibility genes in inter sub-specific hybrids in rice (*Oryza sativa*). Theor Appl Genet (2006) 113:509–517.

Vijaya Kumar (1988). Screening for wide compatibility genes and their linkage with some isozyme markers in rice (*Oryza sativa L.*). Unpublished Ph.D. Thesis, Andhra Pradesh Agricultural University, Hyderabad, India.

Virmani S. S (1998). Hybrid rice research and development in the tropics. In: Virmani, S.S., Siddiq EA, Muralidharan K (eds) Advances in hybrid rice technology. Proceedings of the 3rd international symposium on hybrid rice, 14–16 November1996, Hyderabad, India. International Rice Research Institute, Manila, Philippines, 35–49.

Virmani, S. S., and C. Shinjyo, (1988). Current status of analysis and symbols for male-sterile cytoplasms and fertility-restoring genes. Rice Genet.Newsl.5, 9—15.

Wan. J; S.Yanagihara; H. Kato and H. Ikehashi (1993). Multiple alleles at a new locus causing hybrid sterility between Korean Indica variety and a Javanica variety in rice (*Oryza sativa* L.). Jap. J. Breed ., 43:507–516.

Xie, X. B.; S. Q. Zhang and H. M. Fang (1997). Selection of wide compatible restorer lines and their application in hybrid rice breeding. CRRN, Chinese Rice Res. Newsletter, 5(3): 10-11.

Yang, Z. (1997). Breeding of widely compatible restorer lines (*Oryza sativa L.*) through anther culture. Euphytica. 95: 253-258.

تعريف بعض سلات التوافق العام المعيدة للخصوبة من خلال التهجين الاختبـــارى والمعلمات الجزيئية

رأفت عبداللطيف النمكى

مركز البحوث والتدريب فى الارز – سخا – كفرالشيخ ٣٣٧١٧ – مصر

التهجين بين سلالات متباعدة وراثيا يعطى قوة هجين عالية. اجريت هذة الدراسة بمركز البحوث والتدريب فى الارز – سخا – كفرالشيخ ٣٣٧١٧ – مصر من ٢٠٠٨-الى ٢٠١٠. لتقيم وجود جينات التوافق العام والجينات المعيدة للخصوبة فى سلالات الجيل الخامس (٨٥ سلالة والاباء) الناتجة من التهجين بين صنف معيد للخصوبة (جيزة١٧٨) وصنف توافق عام (Dular). استخدم فى هذه الدراسه ست معلمات جزيئة اربعه منها مرتبطة مع جينات اعادة الخصوبة واثنان مرتبطان مع جينات التوافق العام. بناءا على نتائج المعلمات الجزيئة تم اختيار ١٩ سلالة من بين ٨٥ سلاله. تم اختبار ال١٩ سلالة من خلال تهجينها مع سلالتين عقيمتين زكريا من الطراز الهندى وصنفين(سلالتين) من الطراز الياباتى وذلك لتاكيد نتائج المعلمات الجزيئية.

اكدت النتائج احتواء الصنف جيزة ١٧٨ على جينات التوافق العام فى حين احتوى الصنف Dular على جينات التوافق العام. بالنسبة لسلالات الجيل الخامس اظهرت معظم السلالات احتوائها على كلا من جين التوافق العام وجين اعادة الخصوبة. ايضا اظهرت هجن السلات المنتخبة (ال١٩ سلالة) خصوبة عالية لحبوب اللقاح والسنيبلات مع السلالات الهندية واليابانية. هذة النتائج تشير الى احتواء هذة السلالات على جينات التوافق العام والتى يمكن ان تساعد على انتاج هجن هندية / يابانية بدون اعادة الخصوبة وبالتالى يمكن إستخدمها فى انتاج الهجن الهندية اليابانية بدون عقم حيث اظهرت هذه السلالات قوة هجين عالية لصفة محصول الحبوب بالنبات والتى تراوحت بين١٧,٣١ – ٥٩,٣٤ %.

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