DETECTION OF WIDE COMPATIBILITY AND RESTOEING ABILITY GENES USING MOLECUAR MARKERS IN RICE

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Rice (*Oryza sativa* L.) is one of the most important crops in the world, and used as staple food by more than half of the world population. Hybrid rice showed 15-20% higher yield than the best semi-dwarf inbred varieties (Virmani, 1996; Virmani *et al*., 2003). Hybrid rice technology is considered a viable option to further increase rice yields globally (Yuan, 1994; Janaiah and Hossain, 2000).

Explanation of heterosis for Indica and Japonica subspecies hybrid of rice has been reported, but for a long time, this heterosis has not been directly utilized because of the association effects of low seed setting and poor filling of spikelets, and obvious transgression of plant height and growth duration (Tanaka, 1984; Yuan, 1990). The discovery and exploitation of widely compatible varieties (WCVs) reported by (Ikehashi and Araki, 1984) since the late 1970's, attempts have been made to directly use Indica/Japonica F_1 hybrids by cytoplasmic-genetic male sterility (CMS). The key to this approach is to introduce widely compatible genes into the CMS lines for developing the widely compatible CMS lines. Additionally, strategie is needed to develop widely compatible restorer (WCR) lines, which show strong heterosis, good restoration ability, and compatibility to both Indica and Japonica CMS lines. WCVs are special class of rice germplasm that is able to produce fertile hybrids when crossed to both Indica and Japonica rice (Ikehashi and Araki, 1984). Ikehashi and Araki (1986) also found the *S-5* locus located near C^+ (morphological markers) locus on chromosome 6. This locus has been confirmed in several studies using isozymes (Li *et al*., 1991) and RFLP (Restriction Fragment Length Polymorphism markers) (Liu *et al*., 1992; Zheng *et al*., 1992; Yanagihara *et al*., 1995). Cytoplasmic male sterility (CMS), which causes the production of non-functional pollen and is inherited maternally, is important in commercial hybrid seed production and breeding programs (Kaul, 1988). Fertility-restorer genes are important in the production of hybrid rice when crossed with CMS lines. Although a variable number of restorer genes have been proposed in various restorer lines, one or two dominant restorer alleles (*Rf-3* and *Rf-4*) are usually suggested to be

responsible for the fertility with CMS wild abortive type (Yao *et al*., 1997; Tan *et al.,* 1998). Molecular markers-based genetic maps allow the development and efficient use of indirect selection schemes for germplasm improvement, thereby increasing precision in the manipulation of both qualitative and quantitative traits. Over the past few years, molecular markers using short sequence repeat (SSR) techniques have facilitated the identification of chromosomal regions associated with many complex traits in rice. Therefore, we have conducted this investigation to study the detection of *WC* and *Rf* genes in some segregation lines of rice using SSR as mean markers added selection.

MATERIALS AND METHODS

This investigation was carried out at the experimental farm as well as biotechnology laboratory of Rice Research and Training Center (RRTC), Sakha, Kafr El-Sheikh, Egypt, during the four successive growing seasons from 2003 to 2006.

Plant materials

Twenty promising lines were selected from the fourth segregating generations derived from crosses between, Giza178 x Dular (Dular parent was used as a donor for *WC* genes, while Giza178 parent were used as a donor for *Rf* genes). The selection of these lines depends on the presence of morphological marker C^+ (chromogene for apiculus color) that linked with *WC* gene. The proceedings of the selection are shown in Fig. (1). The selected lines and their parents were screened by molecular techniques to confirm the presence of *Rf* genes as well as *WC* genes.

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.

SSR primers

The simple sequence repeats primer pairs (SSR) namely M2, RM171 and RM3425 have known to be linked with restoring ability alleles, *Rf* 1, *Rf4*, and *Rf3*, respectively to be used for validation. Two primers known to be linked with wide compatibility genes *S-5* and *S-8*, these primers namely; RM253 and RM412, respectively, (Table 1). The primers pairs introduced from Ferments Company Germany and it sequences were directly down-loaded from gramene website (www.gramene.org)

PCR reaction

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 $MgCl_2$, 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 35 cycles, 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 use storage at 4°C. PCR thermocycler machine from Biometra and Applied Bio systems was use.

Electrophoretic analysis

About 1.2 % agarose was used to resolve the low molecular size of DNA molecules. Ethidium bromide 2.5 µl/100 ml was added. Samples run in 1x TBE buffer at 80 volts for were loaded after mixed with 1x loading dye and 3.5 h., DNA ladder 50bp (Ferments Gel photos were taken using Gel Documentation System).

RESULTS AND DISSCUSSION

Five SSR primers were used to detect the wide compatibility (*WC*) and restoring ability (*Rf*) genes. Two of these five SSR primer pairs namely RM253 and RM412, have been reported as markers linked to *S-5* and *S-8 WC* genes, respectively. In addition to three SSR primers pairs namely M2, RM171 and RM3425, which known to be linked with *Rf*-1, *Rf-4*, and *Rf-3*, respectively. RM3425 was selected based on its position near to RG140 which is known to be linked with *Rf1*. DNA extract used as materials from the selected lines for screening the *WC* and *Rf* genes. In addition, the selected lines showed the morphological marker C^+ (chromogene for apiculus color) that linked with *WC* gene (Kumar and Virmani, 1992). The results of gel electrophoresis are presented in Figs. (2-6). In addition, the molecular sizes of bands are presented in Table (2).

Marker linked to Rf genes

M2 primer is a dominant marker that is linked to *Rf*-1 locus on chromosome 1 (Komori and Nitta, 2004). The presence of PCR band reflects the presence of *Rf-1* allele while the absence of the band represents the opposite situation. The results in Fig. (2) showed that the *Rf-1* allele was present in Giza178 and ten selected lines (1, 2, 3, 5, 6, 10, 12, 13, 16, and 20) with band size of 530 base pair (bp) (Table 2), while *Rf-1* allele was absent in Dular parent and other lines. The data obtained from the selected lines and their parents demonstrated the variation among parents and promising lines, which suggested that the parents and promising lines that have the bands may have *Rf-1* gene. This *Rf-1* gene could be used in the future to produce the Japonica hybrids according to Awad-Alla (2006).

RM3425 is a co-dominant marker that linked to *Rf-3* locus on chromosome 1. Also, *Rf-3* is a major restorer gene to CMS wild abortive type (WA) (Majid *et al.*, 2007). The control rice restorer here is Giza178, so any line that produce similar PCR product to Giza178 will be

considered to have *the Rf-3 or Rf-4*. Nine promising lines (1, 2, 3, 7, 8, 10, 11, 12 and 13) have *Rf* allele 2 with band size of 130 bp (Table 2). The results demonstrated that these nine promising lines have *Rf-3* allele 2, and could be used a restorer lines for CMS lines (WA). The data obtained from both selected lines and their parents demonstrated the variation among parents and their selected promising lines. Also, this finding proofs the success of selection among F_4 generation lines to produce lines with *Rf-3* allele. This *Rf-3* allele could be used in the future to produce the Indica hybrids according to Shaoqing Li *et al.* (2005).

RM171 primer is a co-dominant marker that linked to *Rf-4* locus on chromosome 10. In the same time, *Rf-4* is a restorer gene to CMS type *Hong lian* (HL) (Jing *et al.*, 2001; Majid *et al.*, 2007). The results in Fig. (4) showed that ten promising lines (1, 2, 8, 9, 10, 11, 13, 15, 18 and 20) have allele2 with band size of 340 bp, similar to Giza178 while, Dular and other selected lines have *Rf-4* allele1 with band size of 355 bp, (Table 2). These results indicated that these ten promising lines have *Rf-4* allele 2, and could be used as restorer for CMS lines HL type. The data obtained from the selected lines and their parents confirmed the variation among parents and promising lines and also indicating the success to produce of selected lines have the *Rf-4* allele. This *Rf-4* allele could be used in the future as the restorer ability to CMS type (HL) according to Shaoqing *et al.*, (2005).

Marker linked to WC genes

RM253 is a co-dominant marker that linked to *S-5* locus on chromosome 6 (Singh *et al.*, 2006). In the same time, *S-5* a neutral allele, which was used to overcome sterility in F_1 hybrid between Indica/Japonica cross. Dular variety is known to be a WCV, so any line that produce similar PCR product to Dular will be considered to have either *S-5* or *S-8*. The results in Fig. (5) showed that nine promising lines (2, 3, 6, 10, 11, 12, 14, 18 and 20) involved the *S-5* allele1 with band size 140 bp, similar to Dular. These results indicated that these nine promising line have *WC* gene and it could be used to overcome sterility in Indica/Japonica crosses. While Giza178 and the other selected lines represented *S-5* allele 2, with band size of 131 bp (Table 2). The data obtained from both selected lines and their parents indicated the variation among parents and promising lines and also indicating the success to produce of selected lines have the *S-5* allele. These lines could be used to overcome sterility in Indica/Japonica crosses according to (Singh *et al.*, 2006).

RM412 primer is a co-dominant marker that tightly linked to *S-8* locus on chromosome 6 (Wan *et al.*, 1993). In the same time *S-8* is a minor gene for wide compatibility (Singh *et al*, 2006). The results in Fig. (6) showed that nine promising lines (2, 5, 10, 12, 13, 15, 17, 19 and 20) have allele1 similar to the same allele in Dular with band size of 188 bp. While Giza178 and other selected

lines involved of *S-5* allele 2 with band size of 155 bp (Table 2). Also the data obtained from both selected lines and their parents indicated the success to produce of selected lines have the *S-5* allele. These lines could be used to overcome sterility in Indica/Japonica crosses according to Singh *et al.* (2006).

In general, the results discussed earlier confirmed that Dular variety has two alleles (*S-5* and *S-8*) for *WC*. In the same time, Giza178 have three alleles (*Rf-1*, *Rf-3* and *Rf-4*) for *Rf*. However, the selected lines from F_4 generations cleared that the lines number 2 and 10 have five alleles (*S-5*, *S-8*, *Rf-1*, *Rf-3* and *Rf-4*) for both *WC* and *Rf* genes in addition the lines number 12, 13, and 20 have four alleles for *WC* and *Rf* genes, and the lines number 3 and 11 have three alleles including major alleles (*S-5* and *Rf-3*) for *WC* and *Rf*, respectively. These lines might have *WC* and *Rf* genes. Thus, it could be used as widely compatible restorer lines with Indicia and Japonica CMS lines to produce Indica/Japonica hybrid, but it is recommended to have further investigation related to testcross with Indicia and Japonica CMS lines under field condition. Also, the other lines have one major gene only for *WC* or *Rf*. So accumulating more genes for *WC* and *Rf* in single lines would be useful in hybrid production.

SUMMARY

Explanation of heterosis between Indica and Japonica subspecies of rice (*Oryza sativa* L.) has been reported, but for a long time, this heterosis has not been directly utilized because of the associated effects of high sterility in F_1 and poor filling of spikelets, and obvious transgression of plant height and growth duration. Twenty promising lines were selected from the fourth segregating generations which derived from the cross between Giza178 x Dular (Dular is a donor for *WC* genes, while Giza178 is a donor for *Rf* genes). The selection of these F_4 lines depends on the morphological marker C^+ (chromogene for apiculus color) that linked with *WC* gene. DNA was extracted from the selected lines and used for screening the *WC* and *Rf* genes using SSR markers to confirm the presence of restorer genes as well as wide compatibility genes. The results confirmed that Dular variety has two alleles (S-*5* and *S-8*) for *WC*, while, Giza178 has three alleles (*Rf*-1, *Rf*-3 and *Rf*-4) for *Rf*. The selected lines number 2 and 10 have five alleles (*S-5, S-8, Rf*-1, *Rf*-3 and *Rf*-4) for *WC* and *Rf* genes. These lines might have *WC* and *Rf* genes. Thus, they could be used as widely compatible restorer lines with Indicia and Japonica CMS lines to produce Indicia/Japonica hybrids.

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Table (1): Bases sequences of SSR primers used with rice

No.	Alleles	Primer name	Ch	Forward Sequence 5' ⟶ 3'	Reverse Sequence 5' ⟶ 3'
1	*Rf1*	M2	1	TTGGGAGATAAAATGAGGATGTGG	TATAACTCTGGACGACAACGACGG
2	*Rf4*	RM171	10	AACGCGAGGACACGTACTTAC	ACGAGATACGTACGCCTTTG
3	*Rf3*	RM3425	1	AGCAGCAGCAAGAACCCTAG	TTGGTGATCGGTGATGGTC
4	*WCG S-5*	RM253	6	TCCTTCAAGAGTGCAAAACC	GCATTGTCATGTCGAAGCC
5	*WCG S-8*	RM412	6	CACTTGAGAAAGTTAGTGCAGC	CCCAAACACACCCAAATAC

Ch = chromosome number

Table (2): Scoring of five primer pairs for twenty F_4 lines selected from the first population Giza178 x Dular.

Geneotye	M2		RM3425		RM171		RM253		RM412		Rank
	M.S bp	Allele	M.S bp	Allele	M.S bp	Allele	M.S bp	Allele	M.S bp	Allele	
Dular	0	0	146	1	355	1	140	1	188	1	2
L1	530	1	130	2	340	2	131	2	155	2	3
L2	530	1	130	2	340	2	140	1	188	1	5
L3	530	1	130	2	355	1	140	1	155	2	3
L4	-	0	146	1	355	1	-	0	155	2	0
L5	530	1	146	1	-	0	131	2	188	1	2
L6	530	1	146	1	355	1	140	1	155	2	2
L7	-	0	130	2	-	0	-	0	155	2	1
L8	-	0	130	2	340	2	131	2	155	2	2
L9	-	0	146	1	340	2	131	2	155	2	1
L10	530	1	130	2	340	2	140	1	188	1	5
L11	-	0	130	2	340	2	140	1	155	2	3
L12	530	1	130	2	-	0	140	1	188	1	4
L13	530	1	130	2	340	2	131	2	188	1	4
L14	-	0	146	1	355	1	140	1	155	2	1
L15	-	0	146	1	340	2	131	2	188	1	2
L16	530	1	-	0	355	1	131	2	155	2	1
L17	-	0	146	1	-	0	-	0	188	1	1
L18	-	0	-	0	340	2	140	1	155	2	2
L19	-	0	-	0	-	0	131	2	188	1	1
L20	530	1	146	1	340	2	140	1	188	1	4
Giza178	530	1	130	2	340	2	131	2	155	2	3

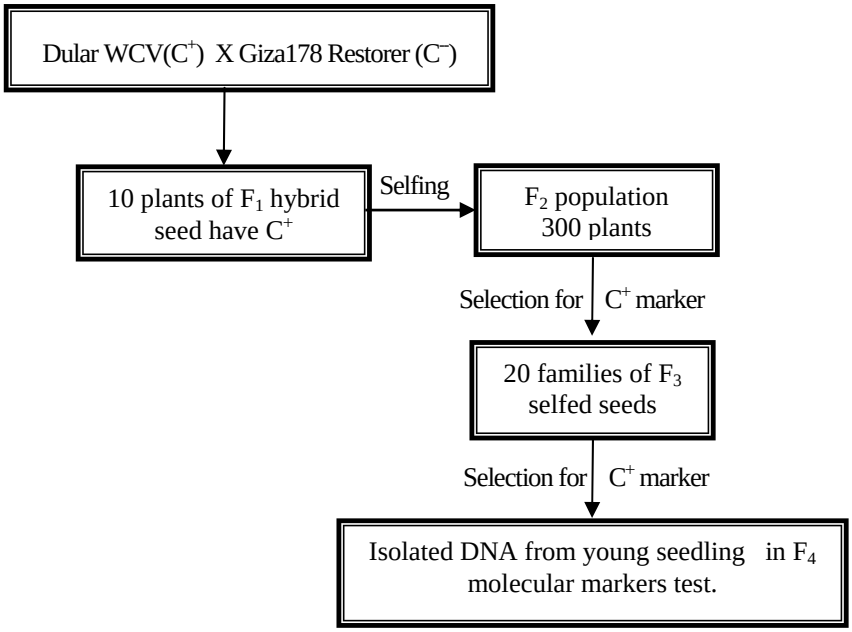

Fig (1): Proceeding of selection depends on chromogen of apiclus color.

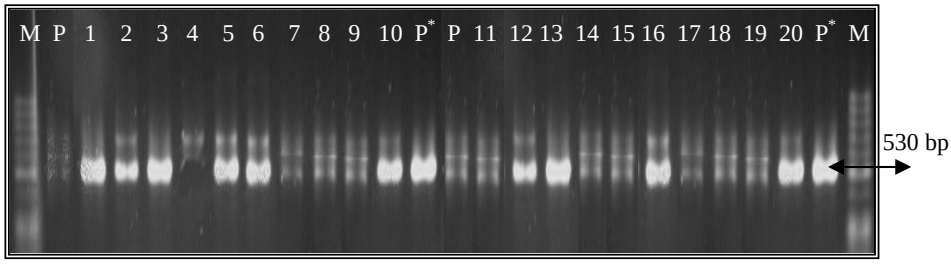

Fig (2): SSR Primer pair M2 with the two parents and 20 lines selected from (Giza178 x Dular).
M: marker P: Dular p*: Giza178 1-20: Selected lines

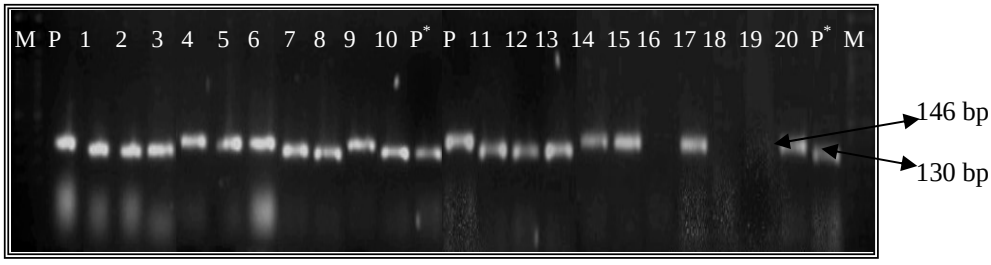

Fig (3): SSR Primer pair RM3425 with the two parents and 20 lines selected from (Giza178 x Dular).
M: marker P: Dular P*: Giza178 1-20: Selected lines

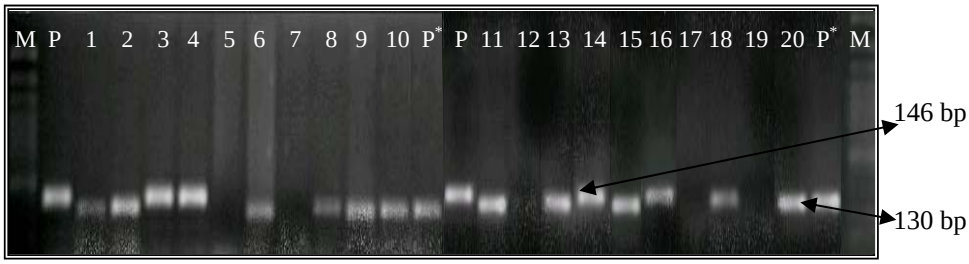

Fig (4): SSR Primer pair RM171 with the two parents and 20 lines selected from (Giza178 x Dular).
M: marker P: Dular P*: Giza178 1-20: Selected lines

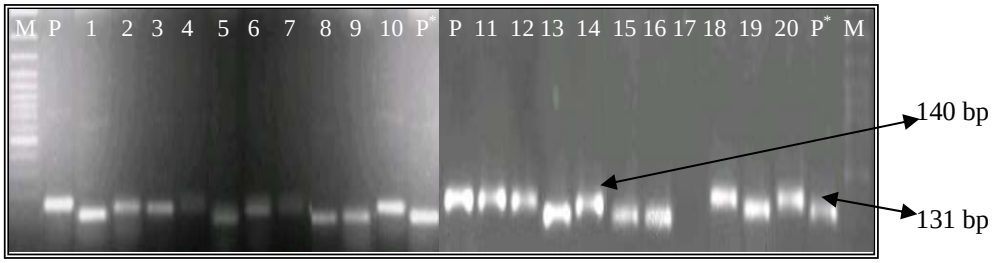

Fig (5): SSR Primer pair RM253 with the two parents and 20 lines selected from (Giza178 x Dular).
M: marker P: Dular P*: Giza178 1-20: Selected lines

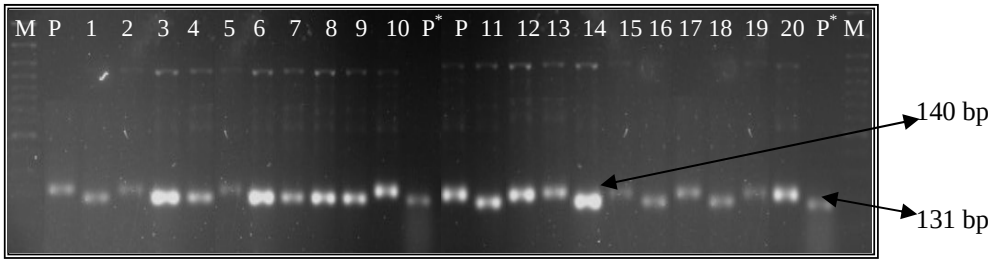

Fig (6): SSR Primer pair RM412 with the two parents and 20 lines selected from (Giza178 x Dular).
M: marker P: Dular P*: Giza178 1-20: Selected lines