

RESEARCH ARTICLE

Genetic analysis of fertility restoration under CGMS system in rice (*Oryza sativa* L.) using three-way test-cross method

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Abstract

We studied the genetics of fertility restoration by producing three-way test cross (TWTC) hybrids involved different combinations of restorers, maintainers and partial restorers of rice. Pollen and spikelet fertility of 16 TWTC hybrids were studied. Six TWTC involving restorer/restorer combinations as male parents produced progenies with fertility levels ranging from complete to zero. No specific ratio of segregation was observed. The crosses involving maintainer/maintainer combinations as male parents showed fully fertile and partial fertile/sterile plants in their progenies. These could be due to nonallelic gene interactions for fertility restoration between the two restorer or maintainer parents, or due to the influence of some modifying genes in the nuclear genome. TWTC involving partial restorer / restorer and partial restorer/partial restorer as the male parents also produced fully fertile and partial fertile/sterile plants suggesting the complex genetics of fertility restoration in rice. There were no previous results depicting the complementation effects of maintainers for fertility restoration.

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Introduction

Achieving self-sufficiency in rice production and maintaining price stability are important objectives in low-income countries, where rice as the staple food provides the basis for national food security and generates employment and income for poor people (Viraktamath *et al.* 2010). Among the plethora of genetic approaches at our disposal for improving rice productivity, hybrid rice has fared well and secured a good track record in uplifting the curse of yield barrier (Anon 2009).

Cytoplasmic genetic male sterility (CGMS) resulting from nuclear-cytoplasmic interaction has been commercially exploited for the production of F₁ hybrid seed in rice. The CGMS system involves three lines, namely a cytoplasmic male sterile (CMS) line, a maintainer line and a restorer line where restorer line (R line) possesses dominant fertility restoring genes. When R line is crossed with the CMS line, it restores fertility in the derived F₁ hybrid. Nonavailability of effective restorers, particularly when novel CMS lines are developed using cytoplasmic donors (wild species) and diverse recipient parents is the major problem in hybrid rice breeding using cytoplasmic male sterility system. Under

such situations, the donor species itself should be explored as a possible restorer. Iso-cytoplasmic restorers have been developed successfully for many CMS lines. CMS sources are distinguished from one another on the basis of the fertility of crosses involving CMS lines and restorers (Eusebio *et al.* 2002).

CMS can be restored by nuclear restorer gene (*Rf* gene). Multiple *Rf* loci are certainly determined by the multiple CGMS systems in the natural populations within a plant species and one or more than one major restoring loci confer complete fertility restoration in a majority of CMS systems. In rice, a variable number of fertility restorer genes can restore complete fertility of a certain CMS line by various restorer lines (Tan *et al.* 2008).

In CGMS systems, restorers are routinely identified by test crossing prospective lines with available CMS lines and evaluating F₁s for pollen and spikelet fertility (Prasad *et al.* 1993). Pollen fertility or spikelet fertility or both have been used as an index to fix the restoration ability of the lines. Precise knowledge of genetics of fertility restoration is an essential prerequisite for efficient restorer breeding programme and the role of monogenic, digenic and trigenic either with or without intergenic interactions is more important in the study of genetics of fertility restoration. Test crosses were earlier used to study the inheritance of fertility restoration.

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Tan *et al.* (2008) stated that if the sterile plants are not observed in a population derived from the test-cross of A/Rf/Rf', the restoring loci between two different restorer lines (Rf and Rf') are thought to be allelic otherwise, the two restoring loci are considered nonallelic. Appearance of partial fertile segregants in crosses with complete restorers suggested the probable role of modifiers in fertility restoration (Govindaraj and Siddiq 1985).

Govindaraj and Virmani (1988) also provided evidence to indicate the presence of different sets of Rf genes in different restorer lines and revealed three types of interaction like epistasis with dominance (12 fertile: three partially fertile / partially sterile: one sterile), epistasis with recessive gene action (9 : 3 : 4), or epistasis with incomplete dominance (9 : 6 : 1). The mode of interaction of the genes of a restorer differed with the CMS line used (Govindaraj and Virmani 1988). Perhaps, complex inheritance and involvement of more than one gene discouraged hybrid rice breeders to use marker-aided selection for fertility restoration (Nas *et al.* 2002).

Sharma *et al.* (2005) performed three-way test-cross (TWTC) and observed that in addition to diversity of restorer gene(s), residual genetic background of restorer lines could also be responsible for their differential restoration ability. He explained the inheritance pattern of three restorer genes on the basis of involvement of two fertility restorer genes (*Rf1* and *Rf2*), whereas the presence of third gene (*Rfe*) was required to allow the expression of *Rf2* since restorer line seemed to possess *rfe* which acted as an inhibitor of *Rf2*.

All the previous studies on genetics of fertility restoration were studied on the inheritance with respect to one restorer per cross combination followed by F₂ analysis. There has been no study to understand the nonallelic complementation of restoration or other kinds of nonallelic genetic interaction among the reported stable restorer lines. In the present study, we have taken a novel approach to use TWTC hybrids involving different combinations of restorers, maintainers and partial restorers, and study their progenies. The present study was attempted to explore the nature and genetics of fertility restoration in a WA-CMS line, COMS23A, by TWTC involving four restorers (C20R, CB87R, CB174R and WGL32100), two partial restorers (BPT5204 and CO43), and four maintainers (ADT43, ASD16, DE2 and CO47).

Materials and methods

Field experiments were carried out to understand the fertility restoration ability (FRA) of the parents, hybrids and the nature of inheritance for fertility restoration through TWTC at the Paddy Breeding Station (PBS), Centre for Plant Breeding and Genetics (CPBG), Tamil Nadu Agricultural University (TNAU), Coimbatore during the year 2008–2010. Details of the materials involved in the present study are provided in table 1.

Evaluation of single crosses

The 11 parents used in this study, including the CMS line, were initially evaluated for pollen and spikelet fertility to confirm their initial fertility levels. Pollen fertility was determined using I-KI staining method (Chaudhary *et al.* 1981) and spikelet fertility was by spikelet counts. Both the pollen and spikelet fertilities were classified as per Standard Evaluation System for rice (IRRI 1996). The 10 male parents along with WA-CMS line (COMS23A) were raised in completely randomized block design with three replications in hybridization block in October 2008.

The progenies of crosses between COMS23A and 10 parents were evaluated for their FRA through pollen and spikelet fertility, and the parents were appropriately classified into restorers, maintainers and partial restorers. About 20 plants in each cross were individually scored for their pollen and spikelet fertility. Based on the percentage of pollen fertility, the male parents were classified as restorer (60–100%), partial restorer (30–59.99%), partial maintainer (1–29.99%) and maintainer (0–0.99%) following IRRI (1986) and Govindaraj and Virmani (1988). Based on spikelet fertility, the male parents were classified as restorer (80–100%), partial restorer (30–79.99%), partial maintainer (1–29.99%), maintainer (0–0.99%) as per Biraj *et al.* (1991).

Single-cross progenies were developed by crossing 10 selected rice genotypes with varying FRA (table 2), to develop TWTC progenies following hand emasculation technique. Sixteen F₁ hybrids obtained from single crosses were raised with their respective parents and CMS line,

Table 1. Details of genotypes and their restoration behaviour.

Genotype	Parentage	Nature of fertility restoration
C20R	Unknown	Complete restoration
CB87R	IR 52280-962-2-3/CB98004	Complete restoration
CB174R	CO43/ASD19	Complete restoration
WGL32100	Divya/BPT5204	Complete restoration
BPT5204	GEB24/T(N)1	Partial restoration
CO43	Dasal/IR20	Partial restoration
ADT43	IR50/improved white ponni	Maintainer
ASD16	ADT31/CO39	Maintainer
DE2	ADT43/jeeragasamba	Maintainer
CO47	IR50/CO43	Maintainer
COMS23A	IR71321A/CB 97003//CB 97003	CMS line

Table 2. Cross combinations of differential restoration.

Nature of FRA	Cross combination
Complete restorer vs complete restorer	C20R/CB87R C20R/CB174R C20R/WGL32100 CB87R/CB174R CB87R/WGL32100 CB174R/WGL32100
Maintainer vs maintainer	ADT43/ASD16 ADT43/DE2 ADT43/CO47 ASD16/DE2 ASD16/CO47 DE2/CO47
Complete restorer vs maintainer	C20R/ADT43 CB87R/ASD16
Partial restorer vs partial restorer	BPT5204/CO43
Partial restorer vs restorer	BPT5204/C20R

COMS23A in May 2009 which constituted the hybridization block for developing three-way crosses.

Development of TWTC progenies

TWTC involved F_1 s from 16 single cross hybrids as male parents and CMS line as the female parent (figure 1). The CMS line was raised in a staggered manner to get synchronized flowering with the F_1 s for efficient crossing. At flowering, the true F_1 s were identified morphologically and confirmed through polymorphic SSR markers and tagged. A total of 16 TWTC progenies were thus obtained.

Evaluation of TWTC progenies

The TWTC progenies were raised by adopting a spacing of 20×20 cm, single seedlings per hill in October 2009. Recommended cultural practices were followed during the crop growth. Before panicle emergence, the primary panicles of all the plants were bagged to avoid contamination from foreign pollen. All the plants in each combination were scored for their FRA through pollen fertility at the time of flowering and spikelet fertility at the time of harvest.

Pollen and spikelet fertility of 16 TWTC progenies were studied to understand the allelism of fertility restoration

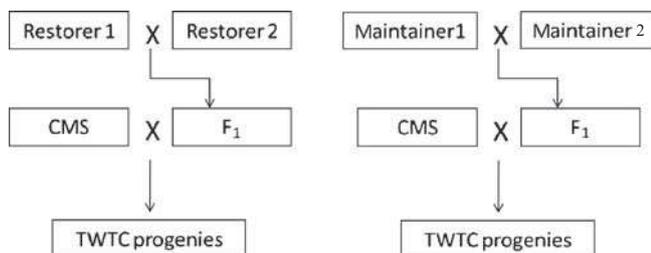


Figure 1. Schematic representation of the development of TWTC progenies.

among the restorers, maintainers and partial restorers. These 16 TWTC progenies were studied under five groups, namely TWTCs involving F_1 s of restorer/restorer, maintainer/maintainer, partial restorer / partial restorer, partial restorer/restorer, restorer/maintainer as the male parents.

Results and discussion

The mean and variance of pollen and spikelet fertility of parents are provided in table 3. The CMS line COMS23A, which was used as tester in this study was completely male sterile with no stainable pollen grains. All the parental varieties under investigation recorded more than 98% and 80% of pollen and spikelet fertility, respectively and therefore indicated that the parents used in this study are good fertile lines without any fertilization barriers.

Testing FRA of parents

The F_1 hybrids resulting from crossing tester COMS23A with the parents were studied to ascertain the restorability of the parents under investigation. The parents were initially test crossed with the CMS line (COMS23A), the resultant hybrids were evaluated for pollen and spikelet fertility and the parents were appropriately classified into restorers, maintainers and partial restorers. The frequency of different classes, mean fertility percentage and variance estimated for pollen and spikelet fertility are provided in table 4. The cross combinations which recorded all fertile plants (i.e. more than 90% and 80% of pollen and spikelet fertility) in the progeny indicated that the parents used were good restorers, namely C20R, CB87R, CB174R and WGL32100.

The cross combinations which produced all sterile plants with a zero per cent pollen and spikelet fertility included male parents ADT43, ASD16, DE2 and CO47, indicating that these parents were absolute maintainers. ADT43 and CO47 are absolute maintainers under wild-abortive (WA) cytoplasm and was converted into new CMS lines, namely, COMS14A and COMS15A (Manonmani *et al.* 2009). Likewise ASD16 was also reported to be a maintainer for WA-CMS line (Wilfred Manuel and Rangaswamy 1993). The single-cross combinations, that recorded partially fertile plants included BPT5204 and CO43 as the pollen parents and the test-cross progenies of those were identified to be partial restorers. The parents BPT5204 and CO43, recorded 27.9 to 38.4 per cent of fertile pollens in concurrence with the previous report (Wilfred Manuel and Rangaswamy 1993). Prasad *et al.* (1993) also had reported that CO43 was a partial restorer for IR58025A. Bharaj *et al.* (1990) reported that a restorer line found to restore completely under a particular CMS line may restore partially under another CMS line possessing the same CMS source. Such differential fertility restoration behaviour of the restorers indicates the complexity of the fertility restoration in inheritance.

Table 3. Mean and variance of pollen and spikelet fertility in parents.

Parent	Pollen fertility per cent		Spikelet fertility per cent	
	Mean	Variance	Mean	Variance
1 C20R	99.0 ± 0.18	0.26	83.2 ± 0.90	2.68
2 CB87R	98.5 ± 0.25	0.83	86.5 ± 0.84	8.62
3 CB174R	98.9 ± 0.27	0.38	91.0 ± 0.86	7.80
4 WGL32100	99.2 ± 0.12	0.22	90.0 ± 0.57	2.93
5 ADT43	99.0 ± 0.28	0.60	84.1 ± 0.98	3.09
6 ASD16	98.7 ± 0.24	0.24	85.2 ± 1.07	4.23
7 DE2	99.7 ± 0.16	0.40	87.2 ± 0.52	1.93
8 CO47	99.8 ± 0.07	0.81	82.9 ± 1.55	6.52
9 BPT5204	99.6 ± 0.10	0.43	90.0 ± 0.87	1.90
10 CO43	99.4 ± 0.21	0.03	86.5 ± 0.80	2.80
11 COMS23A	0.0	0.0	0.0	0.0

Study of allelism of fertility restoration

Pollen and spikelet fertility of restorer/restorer TWTC progenies are provided in table 5. The cross combination, COMS23A/CB87R/WGL32100 was evaluated for both pollen and spikelet fertility, and other TWTC progenies were evaluated for spikelet fertility alone. About 127 to 251 progenies were evaluated in each cross. Progenies of all the six combinations produced sterile plants ranging from 10.39 to 37.32% and partially fertile/sterile plants ranging from 29.88 to 51.97%. The proportion of fully fertile plants observed ranged between very low (20.47%) in the cross COMS23A/C20R/WGL32100(R) and high (52.99%) in COMS23A/CB87R/WGL32100(R). However, in these restorers, fully sterile plants were also observed in the range of 14.62% (COMS23A/C20R/CB87R) to 37.32% (COMS23A/CB174R/WGL32100(R)).

Six TWTC progenies involving maintainer/maintainer cross combinations as the male parents were studied for their segregation for pollen and spikelet fertility and the observations are given in the table 6. The COMS23A/ADT43(B)/CO47(R) was evaluated for both pollen and spikelet fertility and other progenies were evaluated for spikelet fertility alone. The number of plants observed

ranged from 86 to 500 per cross. Except some crosses, total number of sterile plants observed in all the crosses were more than the fertile and partial fertile plants. Number of fully sterile plants ranged from 26 (30.2%) to 444 (88.8%). More partial fertile plants were observed in COMS23A/ASD16 (B)/CO47(B) than fully fertile and sterile plants. The chance of presence of fully fertile plants in the crosses involving maintainers also had been observed in four crosses, and it ranged from 17 plants out of 86 (19.77%) in COMS23A/ASD16(B)/CO47(B) to 74 plants out of 293 (25.3%) in COMS23A/ADT43(B)/CO47(B).

In case of partial restorer–restorer TWTC, namely, COMS23A × (BPT5204 × C20R) only 33 plants were observed to be fertile, 27 plants as sterile and 37 plants as partial fertile. The restoration ability was still reduced among the test-cross progenies involving partial restorer × partial restorer (COMS23A/(BPT5204 × CO43)) when it recorded only 30 plants as fertile and 28 plants as partial and 54 plants as fully sterile.

High yield of F₁ hybrids depend largely upon high pollen and spikelet fertility which is determined by the mode of restorer gene action contributed by the restorer line and its interaction with cytoplasm. Fertility restoration of WA-CMS lines in rice was known to be dominant monogenic

Table 4. Mean and variance of pollen and spikelet fertility in hybrids.

Hybrids	No. of plants	Pollen fertility per cent						Spikelet fertility per cent					
		Frequency				Mean	Variance	Frequency				Mean	Variance
		F	PF	PS	S			F	PF	PS	S		
1 COMS23A/C20R	20	20	–	–	–	82.8 ± 1.64	15.7	14	6	–	–	85.4 ± 0.76	26.2
2 COMS23A/CB87R	20	20	–	–	–	90.2 ± 1.18	20.8	17	3	–	–	88.3 ± 1.45	24.0
3 COMS23A/CB174R	20	20	–	–	–	89.3 ± 1.47	15.7	13	7	–	–	84.7 ± 1.24	32.4
4 COMS23A/WGL32100	20	20	–	–	–	86.9 ± 1.02	17.6	19	1	–	–	82.5 ± 1.39	24.7
5 COMS23A/ADT43	20	–	–	–	20	0	0.00	–	–	–	20	0	0.00
6 COMS23A/ASD16	20	–	–	–	20	0	0.00	–	–	–	20	0	0.00
7 COMS23A/DE2	20	–	–	–	20	0	0.00	–	–	–	20	0	0.00
8 COMS23A/CO47	20	–	–	–	20	0	0.00	–	–	–	20	0	0.00
9 COMS23A/BPT5204	20	9	10	1	–	27.9 ± 0.74	11.8	10	7	3	–	30.2 ± 1.69	16.4
10 COMS23A/CO43	20	12	8	–	–	38.4 ± 1.19	16.9	12	6	2	–	35.4 ± 0.85	33.6

F, fertile; PF, partial fertile; PS, partial sterile; S, sterile.

Table 5. Pollen and spikelet fertility of restorer/restorer TWTC progenies.

	Female	Male (F ₁ s)	Analysis	Observed phenotypic frequencies			
				F	PF + PS	S	Total
1	COMS23A	CB87R/WGL32100	Pollen	133 (53)	75 (29.9)	43 (17.1)	251 (100)
			Spikelet	131 (48.9)	94 (40.7)	24 (10.4)	231(100)
2	COMS23A	C20R/CB87R	Spikelet	67 (51.5)	44 (33.8)	19 (14.6)	130 (100)
3	COMS23A	C20R/CB174R	Spikelet	37 (22.4)	82 (49.7)	46 (27.9)	165 (100)
4	COMS23A	C20R/WGL32100	Spikelet	26 (20.5)	66 (52.0)	35 (27.6)	127 (100)
5	COMS23A	CB87R/CB174R	Spikelet	64 (25.4)	126 (50.4)	60 (24.0)	250 (100)
6	COMS23A	CB174R/WGL32100	Spikelet	35 (24.7)	54 (38.0)	53 (37.3)	142 (100)

F, fertile; PF, partial fertile; PS, partial sterile; S, sterile.

(Huang *et al.* 1987), dominant digenic (Zhou 1983; Li and Zhu 1988) and with different types of gene interactions (Govindaraj and Virmani 1988) governed by more than one major and minor genes. Most of these results emanated from the F₂ population of the single-cross combinations. However, there has been no study to understand the non-allelic complementation of restoration or other kinds of non-allelic genetic interaction among the reported stable restorer lines. The present study has used TWTC hybrids involving different combinations of restorers, maintainers and partial restorers to understand the genetics among the various restorer/maintainer lines.

Restoration behaviour in restorer/restorer TWTC

In the test-cross progenies while crossing restorer with the parents it was expected that a test cross was observed to produce, fertile: sterile plants in the ratio of 1:1 (monogenic) or fertile: partial fertile: partial sterile: sterile plants in the ratio of 1:1:1:1, 1:2:1, 2:1:1, 1:1:2 (digenic and with some epistatic interactions) (Kannan Babu 1995; Motomura *et al.* 2003; Tan *et al.* 2008). However, in this study the F₁ of two restorer lines was used as the pollen parent, the resultant progenies possessed considerable percentage of sterile and partial sterile plants. The occurrence of sterile/partial sterile plants in these combinations could have been due to nonallelic gene interactions for fertility restoration existing between the two restorer parents. This is the first of its kind where F₁s of restorers/maintainers were used as the pollen

parent to study the genetics of fertility restoration and results are of great significance in hybrid rice breeding. The results clearly indicated that a large number of loci were influencing fertility restoration in rice with some being major and other being minor effects. Failure of the results to fit into a definite Mendelian ratio indicates the cumulative influence of few or more modifying genes in the nuclear genome responsible for fertility restoration in rice (table 7).

Recently, a cold-inducible, minor, modifier QTL for the fertility restoration was detected by Ahmadikhah and Alavi (2009) and reported that in cold environment the modifier QTL was responsible for the reduced fertility and deviation of F₂ ratio (15:1). Kumari (1998) reported that the appearance of the partial sterile and fertile plants in crossing of strong restorer lines (IR 36) with CMS lines, and indicated the probable role of modifier genes in the occurrence of fertility restoration that are activated in desirable environmental conditions. Appearance of partial fertile segregants in crosses with complete restorers suggested the probable role of modifiers in fertility restoration (Govindaraj and Siddiq 1985). Two dominant genes Rf_1 and Rf_2 must be present in at least in heterozygous form, for complete pollen fertility in the cytoplasm. Additionally, one or more modifying genes, probably dominants, also must be present in all but in the most favourable environments (Duvick 1956). It is presumed that some lines lack a full complement of modifiers for the gene(s) involved, for example a homozygous line A12-TR is typically a partial restorer in maize for T-cytoplasm. When it was crossed as a female to nonrestorer genotypes or as a male

Table 6. Pollen and spikelet fertility of maintainer/maintainer TWTC progenies.

	Female	Male (F ₁ s)	Analysis	Observed phenotypic frequencies			
				F	PF + PS	S	Total
1	COMS23A	ADT43 / CO47	Pollen	81 (27.4)	73 (24.7)	142 (48.0)	296 (100)
			Spikelet	74 (25.3)	67 (22.9)	152 (51.9)	293 (100)
2	COMS23A	ADT43 / ASD16	Spikelet	78 (41.3)	27 (14.3)	84 (44.4)	189 (100)
3	COMS23A	ADT43 / DE2	Spikelet	0 (0)	35 (7.0)	465 (93.0)	500 (100)
4	COMS23A	ASD16 / DE2	Spikelet	0 (0)	31 (14.8)	179 (85.2)	210 (100)
5	COMS23A	ASD16 / CO47	Spikelet	17 (19.8)	43 (50.0)	26 (30.2)	86 (100)
6	COMS23A	DE2 / CO47	Spikelet	0 (0)	56 (11.2)	444 (88.8)	500 (100)

F, fertile; PF, partial fertile; PS, partial sterile; S, sterile.

Table 7. Pollen and spikelet fertility of other type of TWTC progenies.

Female	Male (F ₁ s)	Analysis	Observed phenotypic frequencies				
			F	PF (PS)	S	Total	
Restorer × maintainer							
1	COMS23A	CB87R/ASD16	PoF	83 (89.3)	10 (10.8)	0 (0)	93 (100)
			SF	80 (86.0)	13 (14.0)	0 (0)	93 (100)
2	COMS23A	C20R/ADT43	SF	0 (0)	9 (39.1)	14 (61.0)	23 (100)
Partial restorer × restorer							
3	COMS23A	BPT5204/C20R	PF	38 (39.2)	35 (36.1)	24 (25.0)	97 (100)
			SF	33 (34.0)	37 (38.1)	27 (28.0)	97 (100)
Partial restorer × partial restorer							
4	COMS23A	BPT5204/CO43	SF	30 (27.0)	28 (25.0)	54 (48.2)	112 (100)

F, fertile; PF, partial fertile; PS, partial sterile; S, sterile; PoF, pollen fertility; SF, spikelet fertility.

to nonrestorer genotypes in T-cytoplasm, it gave hybrids which were fully fertile or fully restored (normal fertility) in all environments (Duvick 1956).

In this study, we could not get full restoration of male fertility in the TWTC, despite both the parents of the pollen plant being good restorers of the same CMS line, but sterility was found to range from 10 to 60%. Allelism analysis of the fertility restorer genes in six three-way test crosses involving four restorers is given in table 8 where recombination frequencies were observed from 14.6% to 32.3% indicating the presence of nonallelic interaction in the restorer gene loci. It has been concluded that this may be due to the lack of full complement of modifiers in both the restorers used. However, Jayasudha and Sharma (2010) indicated that the excessive sterility genes could act as inhibitors of pollen fertility restoration in the F₁ generation. From the allelism test made between the restorers we can conclude that the restorers C20R, CB87R, CB174R and WGL32100 used were nonallelic to each other and they may found to have different kinds of restorer genes. Pande *et al.* (1990) also reported that the variations in the behaviour of fertility restoration indicated either the fertility restoring genes are being different or their performance and expressivity varied with the genotypes of parents' modifiers. The results indicated that some lines apparently carry the modifiers in full complement and hence could fully restore the fertility (Kumari 1998). To some extent, it may seem that hybrid vigour, *per se* etc. causes the fertility of many restorer hybrids to exceed that of their component restorer lines (Duvick 1956).

Pollen and spikelet fertility of maintainer/maintainer TWTC

All the combinations produced about 7 to 50% of partially fertile/sterile progenies instead of expected results i.e., the maintainer will produce sterile progenies while crossing them with their respective CMS line. The attainment of partially fertile/sterile and fertile plants in the three-way test cross involving maintainer/maintainer combinations may be due to the reason that two or more genes with null or minimum phenotypic effect may complement each other and resulted in major perceivable phenotypic effects. There were no previous results depicting the complementation effects of maintainers for fertility restoration trait.

Kazama and Toriyama (2003) studied the complementation of *Rf* gene loci and identified three genes encoding PPR proteins and reported that the pollen with *PPR8-1* major gene restored the fertility and showed normal seed set and not by the minor genes *PPR8-2* or *PPR8-3* genes. From the allelism test made between the maintainers, we can conclude that the maintainers ADT43, ASD16, DE2 and CO47 used were nonallelic to each other and they may have different kinds of maintainer genes with some complementation effects, because in the TWTC progenies involving maintainer/maintainer combinations, some amount of partial fertile and fully fertile plants were observed. In the crosses involving DE2, the progenies did not produce any fertile plants indicating a strong genetic load of maintenance in DE2.

Table 8. Allelism analysis of the fertility restorer genes.

	Crosses	Sterile plants observed	Population size	Recombination frequency (%)	Allelism
1	COMS23A//C20R/CB87R	19	130	14.6	Nonallelic
2	COMS23A//C20R/CB174R	46	165	27.9	Nonallelic
3	COMS23A//C20R/WGL32100	35	127	27.6	Nonallelic
4	COMS23A//CB87R/CB174R	60	250	24.0	Nonallelic
5	COMS23A//CB87R/WGL32100	43	251	17.1	Nonallelic
6	COMS23A//CB174R/WGL32100	53	142	37.3	Nonallelic

Pollen and spikelet fertility of other type of TWTC

The results from these crosses also deviated from the expectations (1:1 (monogenic), 1:1:1:1, 1:2:1, 2:1:1, 1:1:2 (digenic and with some epistatic interactions)) in the test-cross progenies. The results of this study also deviated from the expected and observed results of Anandakumar and Subramaniam (1992) and Tan *et al.* (2008).

In the cross combination involving partial restorer/partial restorer as a male parent, percentage of sterile plants observed was high of about 48.21%. The results support the view that for restorer improvement programme partial restorers can be used as parents.

It was conceived in corn that a gene or genes might have a dominant epistatic effect, such that they could cause fertility even in the presence of *rf₁rf₁*, *rf₂rf₂* or the double recessive homozygotes (Duvick 1956). However, allelism tests of newly discovered restorers have not yet revealed such a gene or genes involved. All restorers tested have been of fully dominant genotypes. A single restorer gene, or the concerted action of several major and minor restorer genes might be able to completely restore the fertility of a male sterile individual. The molecular mechanisms for CMS and restoration differ among species since there are a large number of different complex restoration systems (Schnable and Wise 1998).

Although numerous works on the mapping of fertility restoration genes in rice have been carried out (Zhang *et al.* 1995, 1997, 2002; Yao *et al.* 1997; Jing *et al.* 2001; Zhuang *et al.* 2001, 2002; He *et al.* 2002), because of the complexity of the phenotypic expression, presence of several CMS systems and involvement of many genes and modifiers in the expression of the trait, the fertility restoration genes, especially for WA-CMS have not been exactly identified and localized. Akagi *et al.* (2004) and Wang *et al.* (2006) also explained the complexity of restorer gene loci by analysing the protein products produced by the restorer genes where different genes were responsible for fertility restoration with divergent functions.

As a whole, it has been proved that the four restorers (C20R, CB87R, CB174R and WGL32100R) used in this study possessed different genes for fertility restoration which are nonallelic in nature. The variations in the behaviour of fertility restoration indicated the presence of interallelic interaction between the genes, major or minor modifiers (modifying genes/QTLs) and lack of full complement of modifiers for the gene(s) involved. In some cases the performance and expressivity of the restorer gene(s) might have varied among the genotypes depending on the hybrid vigour. The appearance of sterile plants in TWTC involving restorers are expected only in the case of independent segregation of nonallelic *Rf* genes. In addition to diversity of restorer gene(s), residual genetic background of restorer lines could also be responsible for their different restorability. The complementation of two or more genes with null or minimum effect may be responsible for the appearance of fully fertile plants in TWTC involving two maintainers.

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