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Cyto-Genic Relationship Among Cytoplasmic-Genetic Male Sterile, Maintainer and Restorer Lines of Rice

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The cyto-genic relationship was determined among six of the cytoplasmic-genetic male sterility (cms) rice lines: Zhen Shan 97A, V20A (possessing wild rice *Oryza sativa* f. *spontanea* or *O. perennis* cytoplasm?), Yar Ai Zhao A (possessing Gambiaca cytoplasm). Pankhari 203A (possessing Taichung Native 1 cytoplasm), Wu 10A (possessing Chinsurah Boro II or BT cytoplasm), and MS 577A (possessing *O. sativa* f. *spontanea* cytoplasm). Based on the results of detailed crosses, the six cms lines were classified into four different cyto-sterility systems as follows: Wu 10A and Pankhari 203A (S_1), Zhen Shan 97A/ V20A (S_2), Yar Ai Azhao A (S_3) and MS577A (S_4). The corresponding nuclear genes interacting with a specific cytoplasm to induce sterility in these lines were designated as S_1-rf (for S_1), S_2-rf (for S_2), S_3-rf (for S_3) and S_4-rf (for S_4).

Key words: cytoplasmic male sterility, hybrid rice, nuclear genes, maintainer lines, restorer lines.

During the past decade hybrid rice technology has been developed and used successfully in China (Yuan, 1977) where about 6 million ha (17% of the total rice area) were planted to hybrid rice varieties (Lin and Yuan, 1980: Virmani et al., 1981). These varieties have 20 to 30% yield advantage and wider adaptability in comparison to conventionally-bred rice varieties.

Development of hybrid rice varieties involves primarily the use of cytoplasmic genetic male sterility and fertility restoration system. The role of cytoplasm in causing male sterility in rice was first reported in 1954 (Weeraratne, 1954; Sampath and Mohanty, 1954). Later, Katsuo and Mizushima (1958) and Kitamura (1962a, b) also observed this phenomenon in inter-specific and inter-varietal crosses. Shinjyo and Omura (1966) developed the first cytoplasmic male sterile line in cultivated rice by substituting nuclear genes of a japonica variety, Taichung 65 into the cytoplasm of indica variety Chinsurah Boro II. This line was designated as BT cyto-sterile and the cyto-sterility system was designated as BT. Erickson (1969) and Carnahan et al. (1972) developed cytoplasmic male sterile lines from crosses of an indica variety Birco (PI 279120), with japonica rice varieties of California viz., Calrose, Caloro and Colusa. Watanabe (1971) and Carnahan et al. (1972) developed also cytoplasmic genetic male sterile lines from indica/japonica crosses and *Oryza glaberrima*/ Colusa cross respectively. Moreover, Athwal and Virmani (1972) bred a cytoplasmic male sterile line from an indica/ indica cross, Taichung Native 1/Pankhari 203.

The first cytoplasmic male sterile line used in developing commercial F_1 rice hybrids was developed in China in 1973 from a sterile plant (wild aborted) naturally occurring in a wild rice population (*O. sativa* f. *spontanea* or *O. perennis?*) on Hainan island (Hunan Prov. Agr. Res. Inst., 1977; Yuan, 1977). Subsequently, cytoplasmic male sterile lines have been developed from various accessions of *O. sativa* f. *spontanea*, indica variety Gambiaca (from Africa), and a Chinese variety O-Shan-Tao-Bai (Lin and Yuan, 1980). Currently, about 95% of the area alloted to hybrid rice in China is planted to the hybrids, which are derived from cyto-sterile lines designated as WA (wild aborted) cyto-steriles. However, this situation makes F_1 rice hybrids potentially susceptible to a disease or insect that could be associated with the cytoplasmic factor. It is, therefore, desirable to have diverse sources of cytoplasmic male sterility in rice. The International Rice Research Institute (IRRI) has a collection of cytoplasmic and sterile lines, some of which are derived from different cytoplasmic sources (Virmani et al., 1981). This study was conducted to determine cyto-genic relationship among six of these cms lines to identify different sources of cytoplasmic male sterility.

MATERIALS AND METHODS

Six cytoplasmic-genetic male sterile and their maintainer lines (Table 1) found stable for pollen sterility were used. The following sets of crosses were made: (1) the six cms (A) lines crossed with the six maintainer lines in all possible A/B crosses; (2) the six B lines crossed in all possible B/B crosses; (3) the six A lines crossed with 31 elite lines; and (4) the six B lines crossed with 31 elite lines.

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Table 1. The six cytoplasmic male sterile and their maintainer lines used in the study.

CMS	Maintainer line	Origin	Source of cytoplasm	Remark
Wu 10A	Wu 10B	China	Chinsurah Boro II	Japonica, semidwarf
Pankhari 203A (P 203A)	Pankhari 203B (P 203B)	IRRI	TN1	Indica, photosensitive
Zhen Shan 97A (97A)	Zhen Shan 97B (97B)	China	Wild rice with aborted pollen (*O. sativa* f. *spontanea?*)	Indica, semidwarf
V20A	V20B	China	Wild rice with aborted pollen (*O. sativa* f. *spontanea?*)	Indica, semidwarf
Yar-Ai-Zhao A	Yar-Ai-Zhao B	China	Gambiaca	Indica, semidwarf
577A	577B	Korea	*O. sativa f. spontanea*	Indica, semidwarf

The parents and F_1s of these crosses were grown during 1982 wet season at the rate of 15 plants each in single row plots (3.0 m long). Seedlings were transplanted at the rate of one seedling per hill with a spacing of 30 x 20 cm. Fertilizer was applied at the rate of 80-30-40 (N, P, K) kg per ha. The recommended cultural practices with regard to insect, and weed control were followed.

From each F_1 and parental lines between 40 to 50 spikelets per plant were collected a day before anthesis. Five anthers were collected randomly from the spikelets of each plant to determine pollen fertility. Pollen grains were stained in 1% I_2KI solution and observed under light microscope. Based on their staining behavior and shape they were classified into four categories viz., unstained withered (UW), unstained spherical (US), partially stained round (PSR) and completely stained round (CSR). The first three categories of pollen grains were considered inviable, however, judgment on the viability for the completely stained round pollen grains was based on the spikelet fertility of the bagged panicles.

Spikelet fertility was determined by counting the total number of seed set in proportion to the total number of spikelets in the two primary panicles which had been bagged before flowering.

Mean pollen and spikelet fertility in the F_1s in relation to the parents were used to determine cyto-genic relationships among various cms, maintainer and restorer lines. Linear correlation coefficients were calculated for spikelet fertility in A/B crosses (involving the six cms lines and all the maintaner lines) and A/elite line crosses (involving the cms lines with all the elite lines). The correlation coefficient values for each cms line in relation to the other cms line were used also to determine the cyto-genetic relationship among the cms and maintainer lines.

RESULTS AND DISCUSSION

Pollen and Spikelet Fertility of CMS and Maintainer Lines. Frequency of different categories of pollen grains in the selected cms and maintaner lines indicated that the majority of pollen grains of Zhen Shan 97A (97A), V20A, Yar Ai Zhao A (YAZ A) and Pankhari 203A (P 203A) were unstained withered while majority of pollen grains of Wu 10A and MS 577A were partially stained round to completely stained round (Table 2). P 203A and YAZ A possessed pollen grains of all the four categories but 86 and 90%, respectively, were unstained withered. Pollen grains of the maintainer lines were mostly (96 to 99%) completely stained and round.

Spikelet fertility was zero in 97A, V20A, YAZ A and P 203A possessing mostly unstained pollen grains indicating that unstained pollen grains were inviable. Wu 10A and MS 577A possessing stained and round pollen grains also did not show any seed set on bagged panicles. Apparently, stained round pollen grains of these cyto-sterile lines were also inviable and did not affect fertilization. The cyto-sterile Wu 10A, is derived from BT cyto-sterility system which is known to be gametophytic in nature (Shinjyo, 1969, 1975). Pollen grains of this cms line are known to abort at two nuclear stages of development as compared to 'WA' cyto-steriles in which case the pollen abortion takes place at uni-nuclear stage (Xu, 1982). It appears that stainability reaction and shape of pollen grains in male sterile lines is determined by the stage at which pollen grains abort. The pollen grains aborting at uni-nucleate development stage would be unstained, withered spherical but those aborting at bi-nucleate or later stage would be partially to completely stained and round. By implication, therefore, pollen grains of MS 577A, which are stained normally may be aborting at or after bi-nuclear stage of pollen development. Chaudhary et al. (1981) also derived similar conclusion from their studies on pattern of pollen abortion on some cms lines of rice.

All the six maintainer lines possessing 96 to 99% completely stained round pollen grains showed 85 to 94% spikelet fertility. Their pollen grains were, therefore, viable.

Pollen and Spikelet Fertility in A/B and B/B Crosses. The pollen and spikelet fertility of diallel crosses involving the six cms and their maintainer lines (Table 3) indicated that: (a) the male sterility of Wu 10A was maintained by

Wu 10B and P 203B, the other maintainers acted as partial restorers; (b) the male sterility of 97A and V20A was maintained by all the maintainer lines except YAZ B, the latter behaved as partial restorer; (c) the male sterility of YAZ A was maintained by YAZ B, P 203B, and Wu 10B, the other three maintainer lines behaved as partial restorers; and (d) the male sterility of MS 577A was maintained only by MS 577B, the crosses with other maintainers showed high frequency of completely stained round pollens (like MS 577A) but low spikelet fertility.

These results imply that: (a) the cytoplasmic male sterility system in Wu 10A and P 203A is identical; (b) the cytoplasmic male sterility systems in 97A and V20A is identical; (c) the cytoplasmic male sterility system in YAZ A is different from that of Wu 10A/P 203A, 97A/V20A, and MS 577A; and (d) the cytosterility system of MS 577A is different from Wu 10A/P 203A, 97A/V20A, and YAZ A.

Pollen and spikelet fertility of all possible B/B crosses (Table 4) indicated that: (a) crosses involving 97B, V20B, YAZ B and 577B were normally fertile, without showing reciprocal differences and (b) crosses involving Wu 10B and P 203B used either as maternal or as paternal parent among themselves and with the other maintainer lines showed partial fertility, without showing large and consistent differences between the reciprocal crosses.

Table 2. Frequency of different categories of pollen grains in six cytoplasmic male sterile and maintainer lines of rice.

Line	% Frequency of pollen grain in categories†				Spikelet fertility (%)
	UW	US	PSR	CSR	
Wu 10A	22.8	7.1	25.1	45.0	0
Wu 10B	1.9	2.1	0.1	95.9	87
P 203A	86.5	1.8	4.6	7.1	0
P 203B	0.6	2.0		97.4	94
97A	97.2	2.8			0
97B	0.6	0.5		98.9	92
V20A	97.6	2.4			0
V20B	0.8	0.8		98.4	92
YAZ A	90.4	8.0	0.8	0.8	0
YAZ B	1.3	0.8		97.9	85
577A	1.6	5.0	23.4	70.0	0
577B	0.5	1.5		98.0	91

† UW = Unstained withered.
US = Unstained spherical.
PSR = Partially stained round.
CSR = Completely stained round.

Table 3. Frequency of completely stained round (CSR) pollen (%) and spikelet fertility (%) of F_1s derived from the six cms lines with the maintainer lines in all possible crosses.

CMS Lines	Percent CSR pollen and spikelet fertility† of F_1s involving						Mean
	Wu 10B	P 203	97B	V20B	YAZ B	577B	
Wu 10A	45	33	49	56	84	53	53
	(0)	(1)	(21)	(12)	(27)	(11)	(12.1)
P 203A	56	7	59	56	55	54	47.6
	(2)	(0)	(21)	(22)	(29)	(20)	(15.6)
97A	0	0	0	0	33	0	5.5
	(0)	(0)	(0)	(0)	(13)	(0)	(2.2)
V20A	0	0	0	0	11	0	1.9
	(0)	(0)	(0)	(0)	(10)	(0)	(1.7)
YAZ A	0	2	50	43	1	27	28.7
	(0)	(1)	(25)	(26)	(0)	(16)	(11.2)
577A	50	74	76	70	82	70	70
	(6)	(5)	(6)	(4)	(7)	(0)	(4.8)
Mean	25.2	19.4	38.8	37.5	44.3	42.2	
	(1.3)	(1.2)	(12.4)	(10.7)	(14.2)	(7.8)	

† Spikelet fertility in parenthesis.

Table 4. Frequency of CSR pollen and spikelet fertility (%) in F_1s involving the six maintainer lines in all possible combinations.

CMS Lines	Percent CSR pollen and spikelet fertility† of F_1s involving					
	Wu 10B	P 203B	97B	V20B	YAZ B	577B
Wu 10B	96 (87)	57 (27)	48 (47)	19 (18)	92 (25)	60 (38)
P 203B	49 (28)	97 (94)	45 (46)	31 (25)	37 (26)	44 (20)
97B	76 (41)	47 (49)	99 (92)	99 (89)	99 (84)	97 (70)
V20B	20 (16)	47 (25)	99 (94)	98 (92)	99 (84)	99 (86)
YAZ B	99 (26)	40 (24)	99 (72)	99 (65)	98 (85)	94 (91)
577B	50 (29)	36 (23)	95 (94)	96 (90)	98 (91)	98 (91)

† Spikelet fertility in parenthesis.

Table 5. Linear correlation coefficients for spikelet fertility of sets of A/B crosses involving different cms lines.

CMS Line	P 203A	97A	V20A	YAZ A	577A
Wu 10A	0.92*	0.69	0.69	0.41	0.24
P 203A		0.55	0.55	0.47	- 0.07
97A			1.00**	- 0.44	0.46
V20A				- 0.44	0.46
YAZ A					0.24

** Significant at 1% level; * Significant at 5% level.

Table 6. Linear correlation coefficient for spikelet fertility of sets of A/elite line crosses involving different cms lines.

CMS Line	P 203A	97A	V20A	YAZ A	577A
Wu 10A	0.29	0.06	0.10	0.26	- 0.12
P 203A		0.06	0.04	0.33	0.19
97A			0.99**	0.55**	0.16
V20A				0.48*	0.01
YAZ A					0.03

** Significant at 1% level; * Significant at 5% level.

Partial fertility of crosses involving P 203B and Wu 10B with the other four maintainer lines may be due to gametic development genes identified by Oka (1953, 1954). Therefore, it may be concluded that cytoplasms of the six maintainer lines do not possess factor(s) inducing male sterility.

The values of correlations coefficient for spikelet fertil y of A/B crosses involving the six cms lines and all the maintainer lines are given in Table 5. These values indicated that behavior of Wu 10A and P 203A in crosses with the maintainer lines was highly correlated (r = 0.92); 97A and V20A also showed similar behavior (r = 1). These results further confirmed that cyto-sterility systems in cyto-sterile W 10A and P 203A, and 97A and V20A, were the same. All other possible comparisons among cms lines showed non-significant correlations, hence, the cyto-sterility systems possessed by them sshould be different.

Spikelet Fertility in Crosses of CMS Lines with Elite Lines. Values of correlation coefficients on spikelet fertility of cross involving the cms lines with elite lines are given in Table 6. The results confirmed that the behavior of 97A and V20A in crosses with elite lines was highly correlated (r = 0.99), however, behavior of crosses involving Wu 10A and P 203A did not show significant correlation (r = 0.29). Wu 10A is a japonica line and P 203A is derived from Pankhari 203, the latter is known for giving less fertile hybrids regardless of other parent involved (Engle, 1970; Parmar et al., 1981), therefore, the confounding effect of inter varietal hybrid sterility due to nuclear genes (Oka, 1953; 1954) can not be ruled out. The relationship between Wu 10A and P 203A may be studied further by following up the crosses showing differential behavior in backcross generations where the effects of inter-varietal hybrid sterility are reduced. Correlated behavior of Yar Ai Zhao A with 97A (r = 0.55) and V20A (r = 0.41) may be due to the ability of some elite lines to restore fertility in both cms lines.

Table 7. Proposed cytoplasmic and genotypic constitution of the six cms and their maintainer lines.

CMS/maintainer line	Cytoplasmic constitution	Genotype
Wu 10A	S_1	S_1-rf S_1-rf S_2-rf S_2-rf S_3-rf S_3-rf S_4-Rf S_4-Rf
Wu 10B	N_1	S_1-rf S_1-rf S_2-rf S_2-rf S_3-rf S_3-rf S_4-Rf S_4-Rf
P 203A	S_1	S_1-rf S_1-rf S_2-rf S_2-rf S_3-rf S_3-rf S_4-Rf S_4-Rf
P 203B	N_1	S_1-rf S_1-rf S_2-rf S_2-rf S_3-rf S_3-rf S_4-Rf S_4-Rf
97A	S_2	S_1-Rf S_1-Rf S_2-rf S_2-rf S_3-Rf S_3-Rf S_4-Rf S_4-Rf
97B	N_2	S_1-Rf S_1-Rf S_2-rf S_2-rf S_3-Rf S_3-Rf S_4-Rf S_4-Rf
V20A	S_2	S_1-Rf S_1-Rf S_2-rf S_2-rf S_3-Rf S_3-Rf S_4-Rf S_4-Rf
V20B	N_2	S_1-Rf S_1-Rf S_2-rf S_2-rf S_3-Rf S_3-Rf S_4-Rf S_4-Rf
YAZ A	S_3	S_1-Rf S_1-Rf S_2-Rf S_2-Rf S_3-rf S_3-rf S_4-Rf S_4-Rf
YAZ B	N_3	S_1-Rf S_1-Rf S_2-Rf S_2-Rf S_3-rf S_3-rf S_4-Rf S_4-Rf
577A	S_4	S_1-Rf S_1-Rf S_2-rf S_2-rf S_3-Rf S_3-Rf S_4-rf S_4-rf
577B	N_4	S_1-Rf S_1-Rf S_2-rf S_2-rf S_3-Rf S_3-Rf S_4-rf S_4-rf

On the basis of the foregoing results the six cms lines can be classified into four groups of cyto-steriles viz., Wu 10A/P 203A, 97A/V20A, YAZ A and MS 577A. The cytoplasmic constitution of these four groups may be designated tentatively as below:

Wu 10A/P 203A	S_1	(designated as 'BT' by Shinjyo, 1969)
97A/V20A	S_2	(designated as 'WA' by Yuan, 1977; Lin and Yuan, 1980)
Yar Ai Zhao	S_3	(designated at 'Gam' by Lin and Yuan, 1980)
MS 577A	S_4	(not designated earlier)

Shinjyo (1969) designated the nuclear gene interacting with the 'BT' cytoplasm as 'rf'. Since a number of sterility inducing cytoplasm have been identified, the corresponding nuclear genes interacting with the specific cytoplasmic factor inducing male sterility in these lines may be designated as S_1-rf (for S_1), S_2-rf (for S_2), S_3-rf (for S_3) and S_4-rf (for S_4). Based on these assumptions and the results presented in Table 3 cytoplasmic and genotypic constitution of the six cms and maintaner lines are proposed in Table 7. Studies conducted in China (Zhu, 1979) have also shown that 'WA' cytosterility system is different from 'BT' system.

The use of diverse sources of cytoplasmic male sterility in hybrid rice development should help reduce the possibilities of repeating 'Southern corn blight situation' in rice.

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