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Effect of Male-Sterile Cytoplasm on the Genetic Performance of Agronomic Traits in F_1 Hybrid Rices

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ABSTRACT

Three different male-sterile cytoplasmic lines and their common maintainer 'Zhenshan 97B' and two elite restorer lines were used to study cytoplasmic effects on agronomic trait manifestation *per se* under different nitrogen supply levels. The result showed that cytoplasmic effects could be modified by nitrogen environments. The cytoplasmic effect on grain yield under 150 kg N/ha varied depending on crosses, while it was significantly negative in most crosses under both 60 and 330 kg N/ha. The correlation and path-coefficient analyses suggested that it was expected to improve cytoplasmic effects through reducing maximum tillers and increasing the percentage of productive tillers, leading to increased productive tillers and higher yield in hybrid rice by the aid of cultural practice and genetic transformation. This study also revealed that the same cytoplasm in different combinations had differential effect under the same nitrogen environment, indicating that cytoplasmic effect was produced by interaction of nuclear genes with cytoplasm rather than cytoplasm *per se*. These results indicated the usefulness of evaluating diverse cytoplasmic sources in various nuclear genotypes bred for hybrid rice breeding program. The finding also suggested that negative cytoplasmic effect could be effectively overcome by elite restorer lines through the interaction of nuclear genes with female cytoplasm.

Key words : cytoplasmic effect, environmental modification, homeostasis, hybrid rice, nucleocytoplasmic interaction.

A number of cytoplasmic genetic male sterile lines have been developed in rice. However, the most dependable form of cytoplasmic male-sterile (cms) was obtained from cms-WA (wild abortive) in China and elsewhere (Lin & Wu, 1989; Pradhan et al., 1980). Since the intensive use of a single source of male sterile cytoplasm in developing hybrid varieties was disastrous, as illustrated in the cases of Texas cytoplasm in maize (Hooker, 1974) and Tift cytoplasm in Pearl millet (Pokhriyal et al., 1974), much attention has been paid to the cytoplasmic effect on the performance of hybrid rice and its ecological stability (Lin & Wu, 1989, 1990, 1991, 1996a; Yang & Virmani, 1990). Most results indicated that the male-sterile cytoplasm of WA type had a negative effect on the performance of heterosis, but this effect could be overcome by superior R line and partly regulated by environmental conditions (Lin & Wu, 1989; Ma, 1982; Yang & Virmani, 1990). Therefore, it was considered to be wise to diversify the male sterile cytoplasm when employing the hybrid rice technology (Pradhan et al., 1980; Yang & Virmani, 1990). The objective of this study was to evaluate the effects of three different male sterile cytoplasms on the genetic performance in major agronomic traits of F_1 hybrid rice under different conditions of nitrogen fertilization.

MATERIALS AND METHODS

Three male-sterile cytoplasmic sources, cms-WA, cms-DA and cms-GA, were used to establish the sterile line system by nuclear substitution of more than ten generations with isonuclear genome of their common maintainer Zhenshan 97B (ZB in short) but alien cytoplasms. Each line in the system including ZB was crossed with two elite restorer lines, 'Minhui 63' and 'Che 64', presently used in China, respectively, by employing line × tester design to produce two groups of isonuclear hybrids (A/R and B/R) with different cytoplasms (Table 1).

The formal experiment of a split-split plot design with three replications deploying nitrogen supplies as main-plot, male nuclear type as subplot, and female cytoplasmic type as sub-subplot was carried out at the Experimental Station of Fujian Agricultural University, Fuzhou, P. R China in 1993~1996. Each entry was grown in a single plot with 25 plants spacing 20 cm between rows and 17 cm between plants within the row. Each test row was flanked by Minhui 63 to minimize the border effect.

Ten plants were sampled at random from each entry of each replication for the observation on yield and its components. The analysis of variance was conducted according to the procedure described by Gomez & Gomez (1982).

Cytoplasmic effects on grain yield (Δy), spikelets per

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Table 1. Parental materials used in the experiment to produce hybrid rices (A/R and B/R).

Experimental code	Line and tester†	Origin
WA	Zhenshan 97A	China
DA	D shan 97A	China
GA	G shan 97A	China
ZB	Zhenshan 97B	China
R_1	Minhui 63	China
R_2	Che 64	China

† Zhenshan 97A posses the cytoplasm of wild abortive rice (*Oryza sativa* L. *f. spontanea*), referring to CMS-WA.

D shan 97A has the cytoplasm of Dissi D52/37 rice (*O. sativa* L. subsp. *indica*), referring to CMS-DA.

G shan 97A bears the cytoplasm of Gambliaka Kokum rice (*O. sativa* L. subsp. *indica*), referring to CMS-GA.

Zhenshan 97B is the maintainer of the three A line, referring to ZB, possessing a normal cytoplasm with isonuclear genome of the three A lines.

Minhui 63 (R_1) and Che 64 (R_2) are the two elite restorer lines, showing normal spikelet fertility in all crosses studied.

panicle (ΔX_1), productive tillers per plant (ΔX_2), percentage of filled grain (ΔX_3), 1000 grain weight (ΔX_4), max. number of tillers per plant (ΔX_5), and percentage of productive tiller (ΔX_6) were estimated by comparing A/R combinations (aF_1) and corresponding counterpart [B/R (bF_1)], and testing the significance of the difference (aF_1-bF_1) in pair comparison under the same nitrogen environment with LSD value.

In order to further understand the relationships among cytoplasmic effects on the agronomic traits *per se*, the values of male-sterile cytoplasmic effects on each trait from the average of sampled plants under respective nitrogen treatments were used to calculate genetic correlation and path coefficient by following the procedure described by Ma (1982).

RESULTS AND DISCUSSION

The fact that most of the factors examined including nitrogen supply level, male nuclear type and female cytotype had significant effects on the performance of major agronomic traits in F_1 hybrid rices, indicated that heterotic manifestation was a variable trait depending not only on the parent combination but also on the effect of environmental conditions (Table 2). This finding is consistent with previous reports (Lin & Wu, 1989, 1990, 1991, 1996a, 1996b; Yang & Virmani, 1990). Therefore, we need to consider the role of genotype-environment interaction in breeding F_1 hybrid rices (Yang & Virmani, 1990).

The presence of significant interactions between female cytoplasmic type×male nuclear type, nitrogen environment×female cytoplasmic type and nitrogen environment ×female cytoplasmic type×male nuclear type suggested that cytoplasmic effect was also a variable trait which should be different from the genetic background of nucleo-cytotype and the condition of survival environment. The result of pair comparison in A/R vs B/R cross under the same nitrogen level confirmed that cytoplasmic effect on grain yield showed considerable variation over different nitrogen environments (Table 3). This was also reported in previous research (Lin and Wu, 1989, 1990; Yang and Virmani, 1990). The same cross may show positive effect in certain nitrogen environments but negative in other environments. In this experiment, the cytoplasmic effect on grain yield under 150 kg N/ha varied depending on crosses, while it was significantly negative in most crosses under both 60 and 330 kg N/ha (Table 3). This implies that the cytoplasmic effects on the trait can be modified by different environments. Positive cytoplasmic effect on yield was pronounced in favorable environments, while negative cytoplasmic effects were significant in unfavorable environments.

Cytoplasmic effects on other agronomic traits under consideration also showed considerable variation in different crosses over different nitrogen environments, though not always performing the highest positively significant effects in the condition of 150 kg N/ha. This phenomenon was attributed to the homeostasis in the system of yield and its components including maximum number of tillers per plant and percentage of productive tillers in F_1 hybrid rices. The results of correlation and path-coefficient analyses (Fig. 1) indicated that cytoplasmic effect on grain yield per plant was, to a great extent, correlated with the cytoplasmic effects on the components of grain yield. It appeared that the effects of male-sterile cytoplasms on productive tillers were significantly involved in regulating cytoplasmic effect on grain yield in F_1 hybrid rices, showing the highest direct path coefficient to the effect of male sterile cytoplasm on grain yield. It therefore needs to be considered how the magnitude of positive cytoplasmic effect on productive tillers can be increased under a given environment, consequently leading to increased positive effect of male sterile cytoplasm on grain yield in F_1 hybrid rices. It was found that the direct path-coefficient of cytoplasmic effect on percentage of productive tillers (ΔX_6) to cytoplasmic effect on productive tillers per plant (ΔX_2) was higher than that of cytoplasmic effect on maximum number of tillers per plant (ΔX_5) to ΔX_2 shown in Fig. 1.

The observation suggested that it was effective to increase the percentage of productive tillers under the optimum number of tillers to promote the expression of positive cytoplasm effect on productive tillers per plant by using the technology of cultivation and genetic improvement. This finding has been certified by the fact that super rice breeding and high yield cultivation via fostering a high qualified population with an optimum peak of tillering but higher percentage of productive tillers are under way in China presently.

It is worth mentioning that since the differential reciprocal effect (A/R vs R/B or B/R) resulted from the interaction between the cytoplasm and nuclear genes, the male parent (restorer line), should be also considered in

Table 2. Analysis of variance (split-split-plot design) to determine the influence of main effect and interactions upon the performance of major agronomic traits in F_1 hybrid rices.

Source of variation	Degree of freedom	Mean square (MS)						
		Grain yield (g / plant)	Total spikelets (No. of spikelets / panicle)	Productive tillers (No. of panicle / plant)	Percentage of filled grain (%)	1,000-grain weight (g)	Max. number of tillers (no. of tiller / plant)	Rate of productive tillers (%)
Mainplot								
Replication	2	$0.0235^{ns\dagger}$	0.8430^{ns}	0.0054^{ns}	0.2793^{ns}	0.0013^{ns}	0.1433^{ns}	0.1617^{ns}
Nitrogen (A)	2	325.6294^{**}	310.0510^{**}	31.6092^{**}	777.2610^{**}	4.9643^{**}	1019.5219^{**}	2565.4304^{**}
Error (a)	4	0.1126	5.5963	0.0038	0.0312	0.0024	0.3547	2.1709
Subplot								
Male nucleotype (B)	1	339.3014^{**}	2206.6940^{**}	1.6810^{**}	136.9512^{**}	5.6449^{**}	270.2813^{*}	1602.9235^{**}
A×B	2	3.8862^{**}	28.2810^{**}	0.9703^{**}	10.6738^{**}	1.0551^{**}	31.3404^{**}	69.3127^{**}
Error (b)	6	0.0438	12.4515	0.0335	0.1333	0.0015	0.3307	1.4617
Sub-subplot								
Female cytoplasm (C)	3	30.4346^{**}	292.0250^{**}	2.9463^{**}	8.5312^{**}	5.4186^{**}	108.8483^{**}	377.7401^{**}
A×C	6	9.8248^{**}	595.4302^{**}	1.7677^{**}	9.9305^{**}	0.8837^{**}	6.0638^{**}	35.1538^{**}
B×C	3	93.8901^{**}	2601.8750^{**}	7.8926^{**}	19.2431^{**}	0.0580^{*}	34.3690^{**}	663.7949^{**}
A×B×C	6	9.6251^{**}	257.4953^{**}	1.2462^{**}	4.3708^{**}	0.8209^{**}	9.5110^{**}	45.7014^{**}
Error (c)	36	0.1093	7.2735	0.0239	0.1931	0.0146	0.2686	0.1180

† ns : Nonsignificant at 5% level, *,** : Significant at 5% and 1% level or probability, respectively.

Table 3. Effects of cytoplasms on the genetic performance of major agronomic traits in hybrid rices exposed to different nitrogen environments†.

Crosses	Nitrogen treatment	Grain yield		Total spikelets		Productive tillers		Percentage of filled grains		1,000-grain weight		Max. number of tillers		Rate of productive tillers	
	(kg/ha)	YP	EC	TS	EC	PT	EC	PG	EC	GW	EC	MT	EC	RPT	EC
WA×R_1	60	23.1	-1.9^{**}	130.5	0.1^{ns}	7.6	-0.6^{*}	86.3	0.0	27.1	0.0	21.2	6.2^{**}	5.38	-18.9^{**}
	150	33.7	3.4^{**}	137.2	10.9^{**}	10.5	1.0	84.8	-7.4^{**}	27.6	0.2^{*}	23.0	2.0^{**}	45.7	0.5^{ns}
	330	25.9	-0.6^{*}	130.1	7.5^{**}	8.5	-0.8^{**}	85.5	0.2^{ns}	27.5	0.3^{**}	32.8	6.7^{**}	25.9	-9.7^{**}
DA×R_1	60	25.4	0.4^{ns}	136.6	6.2^{**}	8.7	0.5^{*}	79.2	-7.1^{**}	27.0	-0.1^{ns}	18.0	3.0^{*}	48.3	-6.4^{**}
	150	33.5	3.2^{**}	127.1	0.8^{ns}	10.3	0.8^{**}	92.7	0.5^{ns}	27.6	0.2^{*}	18.9	-2.1^{*}	54.5	9.3^{**}
	330	26.0	-0.5^{ns}	119.0	-3.6^{ns}	9.6	0.3^{*}	85.7	0.4^{ns}	26.6	-0.6^{**}	30.4	4.3^{**}	31.5	-4.1^{**}
GA×R_1	60	22.3	-2.7^{**}	116.6	-13.8^{**}	8.0	-0.2^{ns}	84.4	-1.9^{**}	28.3	1.2^{**}	13.7	-1.3^{**}	58.4	3.7^{**}
	150	28.2	-2.1^{**}	118.2	-8.1^{**}	9.5	0.0	92.0	-0.2^{ns}	27.3	-0.1^{ns}	15.7	-5.3^{**}	60.5	15.3^{**}
	330	24.2	-2.3^{**}	114.3	-8.3^{**}	9.6	0.3^{*}	82.6	-2.7^{**}	26.7	-0.5^{**}	21.6	-4.5^{**}	44.4	8.8^{**}
ZB×R_1	60	25.0		130.4		8.2		86.3		27.1		15.0		54.7	
	150	30.3		126.3		9.5		92.2		27.4		21.0		45.2	
	330	26.5		122.6		9.3		85.3		27.2		26.1		35.6	
WA×R_2	60	18.0	-4.3^{**}	106.6	-2.0^{**}	7.5	-0.5^{*}	84.9	-2.6^{**}	27.0	0.1^{ns}	19.1	0.9^{*}	39.3	-4.7^{**}
	150	27.8	0.6^{*}	113.8	-3.9^{ns}	10.0	0.5^{*}	90.1	-0.4^{ns}	27.0	0.1^{ns}	25.7	0.7^{ns}	38.9	0.9^{ns}
	330	19.9	-5.4^{**}	101.1	-14.4^{**}	8.9	-0.8^{**}	83.4	-2.1^{**}	26.5	0.1^{ns}	35.7	-0.1^{ns}	24.9	-2.1^{**}
DA×R_2	60	17.7	-4.6^{**}	107.8	-10.8^{**}	7.5	-0.5^{*}	83.3	-4.2^{**}	26.3	-0.6^{**}	18.2	0.0	41.2	-2.8^{**}
	150	27.4	0.2^{ns}	118.5	0.8^{ns}	9.7	0.2^{ns}	87.0	-3.5^{**}	27.4	0.5^{**}	24.4	-0.6^{ns}	39.8	1.8^{**}
	330	23.9	-1.4^{**}	112.8	-2.7^{ns}	9.9	0.2^{ns}	81.4	-4.1^{**}	26.2	-0.2^{*}	32.2	-3.6^{**}	30.7	3.6^{**}
GA×R_2	60	19.8	-2.5^{**}	114.2	-4.4^{ns}	7.6	-0.4^{*}	87.7	0.2^{ns}	25.9	-1.0^{**}	17.9	-0.3^{ns}	42.5	-1.5^{**}
	150	26.1	-1.1^{**}	114.1	-3.6^{ns}	10.2	0.7^{*}	86.7	-3.8^{ns}	25.8	-1.1^{**}	21.9	-3.1^{**}	46.6	-8.6^{**}
	330	17.7	-7.6^{**}	113.4	-2.1^{ns}	7.8	-1.9^{**}	79.2	-6.3^{**}	25.3	-1.1^{**}	31.2	-4.6^{**}	25.0	-2.1^{**}
ZB×R_2	60	22.3		118.6		8.0		87.5		26.9		18.2		44.0	
	150	27.2		117.7		9.5		90.5		26.9		25.0		38.0	
	330	25.3		115.5		9.7		85.5		26.4		35.8		27.1	
LSD(0.05)			0.55		4.54		0.26		0.73		0.20		0.86		0.57
LSD(0.01)			0.74		6.00		0.74		0.98		0.27		1.15		0.76

† The data were the average of three replications. EC; Effects of cytoplasms, YS; Yield per plant (g/plant), TS; Total spikelets per panicle, PT; Productive tillers per plant, PG; Percentage of filled grains (%), GW;1,000-grain weight (g), MT; Max. number of tillers per plant, RPT; Rate of productive tillers (%), ns; Non significant at 5% level, *,**; Significant at 5% and 1% levels, respectively.

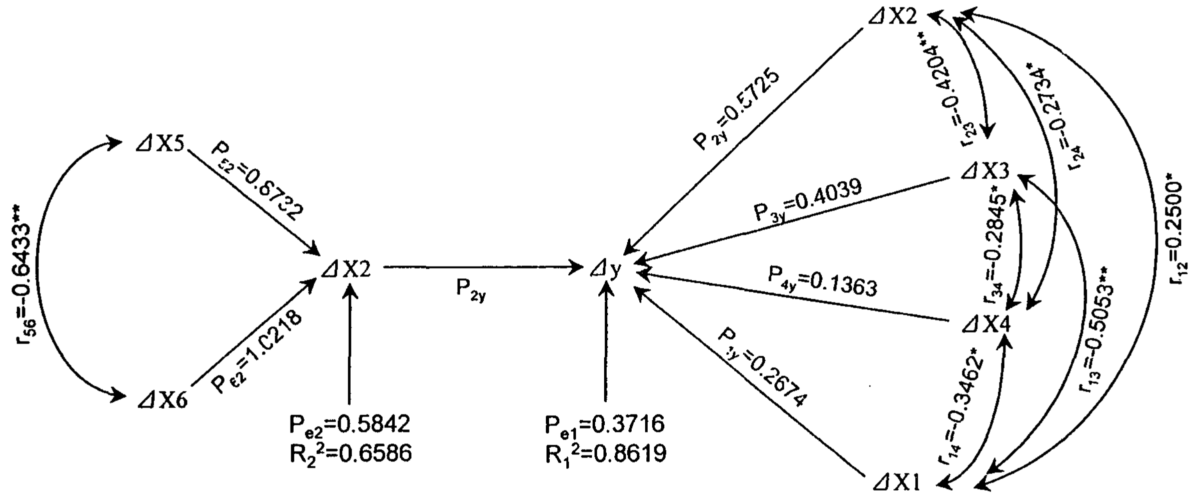

Fig. 1. Diagramic representation of factors influencing male-sterile cytoplasmic effect on grain yield per plant in F_1 hybrid rices.

Δy, Value of cytoplasmic effect on grain yield. ΔX_1, Value of cytoplasmic effect on spikelets per panicle. ΔX_2, Value of cytoplasmic effect on productive tillers per plant. ΔX_3, Value of cytoplasmic effect on percentage of filled grain. ΔX_4, Value of cytoplasmic effect on 1,000-grain weight. ΔX_5, Value of cytoplasmic effect on max. number of tillers per plant. ΔX_6, Value of cytoplasmic effect on percentage of productive tiller. P_{1y}, P_{2y}, P_{3y}, $P_{\Delta y}$, P_{52} and P_{62} are direct path-coefficients. Indirect path-coefficients can be calculated by the formula of r_{ij} P_{iy} P_{jy} ($i>j$). P_{e1} and P_{e2} are residual effects on Δy and ΔX_2, respectively.

Degree of freedom (df)=n−2=58, $r_{0.05}$=0.254, $r_{0.01}$=0.347, *,** ; significant at 5% and 1% levels, respectively.

evaluation. The result of this study illustrates well that R_1 (Minhui 63) appeared in more cases to be positively significant difference in pair comparison between A/R and B/R than R_2 (Che 64) when used as male parent under the same condition of nitrogen environment shown in Table 3. The differential effect of a cytoplasm in different combinations indicated that cytoplasmic effects are produced by the interaction of nuclear genes with cytoplasm rather than cytoplasm *per se*. This implies that evaluation of cytoplasmic sources with various nuclear genotypes is necessary in hybrid rice breeding, and at the same time it is also suggested that negative cytoplasmic effect could be overcome by elite restorer lines via the nucleo-cytoplasmic interactions. Therefore, it is crucial to develop excellent R lines in three line hybrid rice breeding.

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