Development of isonuclear male sterile lines with four different cytoplasmic backgrounds in rice

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For the first time in rice, four cytoplasmic genetic male sterile (CMS) lines with different cytoplasmic backgrounds, namely, Wild abortive, *Oryza perennis* (through conversion), Kalinga I, and Lalruma (through *indica/indica* hybridization) were developed by repeated backcrossing with a common isonuclear maintainer, cultivar (cv.) Krishna. These four new CMS lines with a common nuclear background were confirmed to be cytoplasmically different from one another.

THE importance of the use of cytoplasmic genetic male sterile (CMS) line in the development of hybrid rice in a three-line system is well known to the breeders. With this objective in view, the first CMS line in a self-pollinated crop like rice was developed in China from a male sterile plant (*Oryza sativa* f. *spontanea*), designated as Wild abortive (WA) (ref. 1). Subsequently, even though more CMS lines from different sources were developed in China[2], none of these were found suitable for the diverse climatic conditions in countries outside China. Therefore, a number of male sterile lines have been developed from various accessions of cultivated and wild rices at the International Rice Research Institute (IRRI), Philippines, and elsewhere[2–5]. However, the cytoplasmic genetic male sterile system used extensively to develop commercial hybrid rice in China and India has been mainly developed from a wild abortive (WA) cytoplasmic source. Intensive use of a single source of male sterile cytoplasm in developing hybrids is considered disastrous, as was the case with Texas cytoplasm in maize[6]. Virtually all major crops, including rice, have a narrow genetic base, and consequently are vulnerable to virulent diseases, insects or erratic climatic factors.

Therefore, it was considered important to diversify the sources of male sterile cytoplasm and to develop new CMS lines of WA cytoplasm for hybrid rice breeding in India. However, lack of efficient techniques to characterize CMS sources as well as nonavailability of effective restorers for CMS lines, are two major problems. With this objective in view, four CMS lines with different cytoplasmic backgrounds (Wild abortive, *O. perennis*, Kalinga I, and Lalruma) were developed by repeated backcrossing with a common isonuclear maintainer cv. Krishna, and characterization of the cytoplasmic sources was established by interaction of these CMS lines with elite lines.

The two CMS lines Krishna A with WA cytoplasm and Krishna A with *O. perennis* cytoplasm were developed through conversion involving the CMS lines V20A (WA) and IR 66707A (*O. perennis*), respectively, by recurrent backcrossing to a common isonuclear maintainer, Krishna (as the pollen parent). The CMS line Krishna A with Kalinga I cytoplasm was developed through *indica/indica* hybridization[7], and by repeated backcrossing with the pollen parent, Krishna. However, for the development of the Krishna CMS line with Lalruma (*indica*) cytoplasm, 36 crosses and their reciprocals were attempted using land races and improved high-yielding varieties. Completely sterile plants of the F_1 cross Lalruma/Krishna, which showed high reciprocal differences for pollen and spikelet sterility, were selected and backcrossed to their respective male and female parents. The backcross population (Lalruma/Krishna)/Krishna which showed complete pollen and spikelet sterility in BC_1 was further backcrossed to the recurrent male parent, Krishna, up to the sixth backcross generation (BC_6).

To understand the nature of these four sterile cytoplasms, these were testcrossed with three maintainers, namely, V20B (maintainer of WA), Yar-Ai-ZhaoB (maintainer of Gambiaca), and MS 577B (maintainer of *O. sativa* f. *spontanea*) and five lowland elite lines, namely, IR 48725-B-B-120, SPR 7210-1-3, NDR 30074, TTB 150-61-2-1, and R 657-93-869. All the crosses were evaluated for their pollen and spikelet sterility/fertility. Pollen grains were stained with iodine-potassium iodide solution (I-KI) and examined under the microscope for determining the pollen sterility. Morphological features like plant height, flowering duration, ear bearing tillers (EBT), panicle length and exsertion, and number of spikelet per panicle were recorded for 20 plants.

The F_1 crosses of V20A/Krishna, and IR 66707A/Krishna showed complete pollen sterility with no seed set. Completely sterile F_1 plants of these two crosses were utilized for the development of new CMS lines Krishna A with wild abortive cytoplasm and Krishna A with *O. perennis* cytoplasms by repeated backcrossing with a common isonuclear maintainer, Krishna. Complete pollen sterility with no seed set was noticed in all the backcross generations (BC_1 to BC_6).

The F_1 cross Kalinga I/Krishna showed complete sterility with high reciprocal difference[7]. The sterile F_1 plants were repeatedly backcrossed to the recurrent male parent, Krishna[7], up to the BC_6 generation and then complete sterile Krishna A with Kalinga I cytoplasm were developed, which showed cold tolerance during seedling stage.

Out of the 36 crosses of *indica* (land races)/*indica* (improved high yielding varieties), complete pollen and spikelet sterility was observed in the F_1 plants of cross Lalruma/Krishna with high reciprocal difference, i.e. its

Table 1. Pollen sterility and spikelet fertility percentage in parents, F_1, backcrosses and reciprocal of the CMS line Krishna A derived from Lalruma (*indica* rice)

Parents and cross combination	Generation	Pollen sterility (%)	Spikelet fertility (%)
Lalruma (*indica*)	P_1	5.8	92.1
Krishna (*indica*)	P_2	4.1	95.2
Lalruma/Krishna	F_1	100	0
Krishna/Lalruma	F_1	3.9	96.3
(Lalruma/Krishna)/Lalruma	BC_1P_1	29.8	67.8
(Lalruma/Krishna)/Krishna	BC_1P_2	100	0
(Lalruma/Krishna)/Krishna	BC_2P_2	100	0
	BC_3P_2	100	0
	BC_4P_2	100	0
	BC_5P_2	100	0
	BC_6P_2	100	0

Table 2. Pollen sterility and spikelet fertility (%) along with other characters of four CMS lines of Krishna A

CMS line	PH (cm)	EBT	FD (days)	PL (cm)	PE (cm)	PS (%)	SPP no.	SF (%)
Krishna A (WA)	56	23	89	21.6	11.0 (50.9%)	100	102	0
Krishna A (*O. perennis*)	49	24	97	20.3	8.6 (42.9%)	100	90	0
Krishna A (Kalinga I)	59	32	90	21.3	11.9 (55.9%)	100	109	0
Krishna A (Lalruma)	60	29	94	20.9	10.6 (50.7%)	100	112	0

Value of panicle exsertion in percentage in parenthesis.
PH, plant height; EBT, ear bearing tillers; FD, 50% flowering duration; PL, panicle length; PE, panicle exsertion; PS, pollen sterility; SPP, spikelet per panicle; SF, spikelet fertility.

reciprocal cross Krishna/Lalruma showed high fertility (both pollen and spikelet) (Table 1). Similarly, high F_1 sterility was observed in most of the *indica/indica* crosses where Pankhari 203 was involved as the male parent[8].

Completely sterile F_1 plants of Lalruma/Krishna were backcrossed to both its parents. In the backcross BC_1P_1, sterility decreased, when the female parent Lalruma was used as the recurrent male parent. However, when the male parent Krishna was backcrossed to the F_1 plants as the recurrent parent, complete sterility was observed in BC_1P_2 (Table 1). Since all progenies in BC_1P_2 generation of the backcross (Lalruma/Krishna)/Krishna were found to be completely sterile, they were further backcrossed (up to BC_6) to the recurrent male parent Krishna, in order to develop Krishna-male-sterile-line with Lalruma cytoplasm. Results of these reciprocal backcrosses indicated the effects of cytoplasm in inducing male sterility, and have confirmed that Krishna with fertile cytoplasm maintains the sterility, while, Lalruma with sterile cytoplasm restores fertility. With the development of Krishna A with Lalruma cytoplasm, four CMS lines were developed possessing different cytoplasms, in the genetic background of a single rice cv., Krishna. This is the first report of development of isonuclear CMS lines possessing four different cytoplasmic backgrounds.

All the four CMS lines of Krishna A with different cytoplasmic sources (WA, *O. perennis*, Kalinga I, Lalruma) are dwarf in stature, of medium duration (Table 2), and have white shrivelled anthers with 80–90% unstained, withered, sterile (UWS) pollen grains; and the rest with unstained, spherical, sterile (USS), pollen grains.

To study the diversity among the four sterile cytoplasms, Krishna CMS lines were testcrossed with the three maintainers, namely, V20B, Yar-Ai-ZhaoB, MS 577B and with five lowland elite lines (Table 3). Results indicated that the male sterility of all these four CMS lines could be maintained effectively by V20B, Yar-Ai-ZhaoB, and MS 577B.

On the other hand, the results for the elite lines were: (i) IR 48725-B-B-120; while it restored the fertility effectively (> 80% spikelet fertility) in Krishna A (WA

Table 3. Pollen and spikelet fertility (%) in testcrosses between CMS (A)/maintainer (B) lines, and CMS (A)/lowland elite lines

Genotype	Krishna A (WA)	Krishna A (*O. perennis*)	Krishna A (Kalinga I)	Krishna A (Lalruma)
Maintainers				
V20B	0	0	0	0
	(0)	(0)	(0)	(0)
Yar-Ali-ZhaoB	0	0	0	0
	(0)	(0)	(0)	(0)
MS 577B	0	0	0	0
	(0)	(0)	(0)	(0)
Lowland elite lines				
IR 48725 B-B-120	86.1	0	76.1	69.2
	(82.8)	(0)	(74.9)	(66.7)
SPR 7210-1-3	92.9	0	0	0
	(88.3)	(0)	(0)	(0)
NDR 30074	0	0	0	34.3
	(0)	(0)	(0)	(27.2)
TTB 150-61-2-1	54.2	0	63.8	89.7
	(40.7)	(0)	(53.9)	(81.2)
R 657-93-869	60.3	0	0	74.2
	(42.2)	(0)	(0)	(56.2)

Value of spikelet fertility percentage in parenthesis.

cytoplasm) and partially (< 75% spikelet fertility) in Krishna A (Kalinga I cytoplasm), and Krishna A (Lalruma cytoplasm), it maintained the sterility effectively in Krishna A (*O. perennis* cytoplasm). (ii) SPR 7210-1-3; whereas it restored the fertility, effectively in Krishna A (WA cytoplasm), it maintained the sterility of the other three CMS lines, Krishna A (*O. perennis* cytoplasm), Krishna A (Kalinga I cytoplasm), and Krishna A (Lalruma cytoplasm). (iii) NDR 30074; maintained the male sterility in Krishna A (WA cytoplasm), Krishna A (*O. perennis*), and Krishna A (Kalinga I cytoplasm), but was a partial fertility restorer of Krishna A (Lalruma cytoplasm). (iv) TTB 150-61-2-1; while it restored the fertility effectively in Krishna A (Lalruma cytoplasm), it was a partial restorer of Krishna A (WA cytoplasm), and Krishna A (Kalinga I cytoplasm), and maintained the male sterility in Krishna A (*O. perennis* cytoplasm). (v) R657-93-869; maintained effectively the male sterility of the two cytoplasmic sources, Krishna A (*O. perennis*) and Krishna A (Kalinga I), but restored the fertility partially in other two male sterile lines, Krishna A (WA cytoplasm) and Krishna A (Lalruma cytoplasm). However, none of the 5 lowland elite lines could restore the fertility of the Krishna A (*O. perennis* cytoplasm).

On the basis of our studies, since the four CMS lines interacted differently with each of the five lowland elite lines, despite their common nuclear background, we have concluded that the cytoplasmic factor(s), inducing male sterility, in these CMS lines were different from one another.

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