Development of New Cytoplasmic Male Sterile Lines with Good Flowering Behavior for Hybrid Rice Breeding

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Abstract

Intensive use of single source of male sterile cytoplasm (i.e. wild abortive or WA) in developing hybrid rice might lead to genetic vulnerability associated with susceptibility to pests and diseases. The research aim was to develop new cytoplasmic genetic male sterile lines (CMS) from 3 different cytoplasmic sources (WA, Gambiaca and Kalinga). Anther culture and successive backcrosses were conducted to develop new CMS. The screening of bacterial leaf blight to each CMS was done through artificial inoculation using three isolates i.e. pathotype III, IV and VIII. The results showed that combination of anther culture and backcrosses obtained 6 CMS lines of WA type, 3 CMS lines of Kalinga type, and 1 CMS line of Gambiaca type. The lines showed complete and stable sterility from first to fifth backcross generations. Some of them also showed resistance to certain *Xanthomonas oryzae* pathotypes. The new CMS lines with WA, Kalinga and Gambiaca cytoplasm were early in maturity with flowering behavior better than IR58025A (check CMS), such as larger stigma, higher stigma exertion, wider angle and longer duration of glume opening during anthesis. The accumulation of good flowering behavior increased seed set of the new CMS lines yielded from outcrossings. It ranged from 4.75–25.9%, meanwhile IR58025A seed set only reached 2.98%. Corellation analysis showed high positive and significant value between seed set of the new CMS lines and stigma width (r = 0.44*), stigma exertion (r = 0.54*) and angle of glume opening during anthesis (r = 0.42*), respectively. The seed set was also affected by some flower characters of the maintainer lines such as filament length and angle of glume opening during anthesis. The results indicated that the new CMS lines with diferent cytoplasm sources could be used in hybrid rice breeding.

Keywords: rice, cytoplasmic male sterility, flowering behavior, resistance, bacterial leaf blight

Introduction

Hybrid rice is able to increase rice productivity about 15-20% higher than the best commercial inbred varieties. Currently, hybrid rice technology is used in a large scale cultivation in several Asian countries. Hybrid rice covers more than 50% of rice total area in China (You *et al*., 2006). Three line system is generally used in Indonesia. This system needs three parental lines: cytoplasmic male sterile line (CMS/A line), maintainer line (B line) and restorer line (R line). Hybrid is the first filial generation of a cross between cytoplasmic male sterile line as female parent and restorer lines as male parent. The hybrid often exhibits heterosis or hybrid vigor, where hybrid progeny shows superior growth characteristics relative to one of the parental lines (Eckards, 2006). The commercial hybrid refers to a superior F_1, which not only performs better than its parents but also shows significant yield superiority over the best high yielding inbred variety of similar growth duration (Virmani & Kumar, 2004). Based on those, superior parental lines i.e. CMS needs to be developed.

CMS as female parent is a key factor for hybrid rice breeding. CMS is a condition under which a plant is unable to produce functional pollen (Eckardt, 2006). This line provides a valuable tool in production of hybrid seed in self-pollinating crop species including rice. Development of CMS in three line systems of hybrid rice breeding faces problems among others instability of pollen sterility, flower behaviour not fully supporting outcrossing rate, and susceptibility to pests and diseases. Unstable pollen sterility causes difficulty in achieving high heterosis of the hybrid rice and its seed production. The low seed yield has been implicated in higher price of the hybrid seed. *Wild Abortive* (WA) CMS is mainly used in hybrid rice breeding in Indonesia (Suwarno *et al*., 2003) and elsewhere. This might lead to genetic vulnerability associated with susceptibility to pests and diseases (Li *et al*., 2007). Therefore, breeding of new CMS having stable pollen sterility derived from diverse cytoplasm sources and resistant to pests and diseases are warranted.

Commercial CMS should have good outcrossing rate, complete and stable sterile pollen, easy restorability, good and wide combining ability with any restorer lines, short plant, good exsertion of panicle, more than 70% of stigma exsertion, good quality, resistant to pests and diseases and adapted to target environment (Yuan *et al*., 2003). Dewi *et al*. (2007) identified 19 doubled haploid of maintainer line candidates through anther culture from F_1 of crossing between introduced CMS and donor lines resistant to bacterial leaf blight. The doubled haploid lines are potential maintainers to be converted to CMS because these lines were homozigous and having male sterile gene with normal cytoplasm. Rumanti *et al*. (2009) tested 48 F_1s, derived from testcross between cytoplasmic male sterile sources with those doubled-haploid maintainer lines, and 14 F_1s were completely sterile (100 % pollen sterility). The objectives of this research were to develop stable and completely sterile CMS lines from three different cytoplasmic sources, to evaluate resistance of the new CMS to bacterial leaf blight and study of flower behavior of the new CMS that support outcrossing ability.

Materials and Methods

Development of cytoplasmic male sterile lines through succesive backcross

Genetic material in this research were backcross population (F_1BC_n) between CMS candidates with their maintainer doubled haploid lines. CMS from International Rice Research Institute (IRRI) were used as cytoplasm donors, i.e. 3 CMS of wild abortive type (IR58025A, IR62829A, dan IR68897A), one Gambiaca type (IR80154A) and one Kalinga type (IR80156A). Succesive backcrosses were done 5 times. Each CMS candidate or female parent (F_1BC_n) was planted in 2 rows with 12 plants each, while male parent or their maintainer were planted in 3 rows with 12 plants each. Flowering syncronization was achieved by staggered planting of male parents in a 3-day intervals. Selection was based on pollen sterility in each F_1BC_n. Observation were done on each F_1BC_n generation for pollen sterility (%) (IRRI, 2002).

Resistance evaluation of the new cytoplamic male sterile lines to bacterial leaf blight

Ten new CMS' were planted in the field in 2 rows with spacing of 20 x 20 cm. Plants were inoculated with 10^8 cfu concentration of *Xanthomonas oryzae* pathotype using artificial method (leaf cutting/clipping). Disease severity was observed following modified IRRI method (IRRI, 2002) when susceptible check achieved 90% severity. Score of disease severity is shown in Table 1.

Table 1. Score of disease severity

Scale	Ratio of lesio length and leaf length (%)	Reaction
1	1-5	R (Resistant)
3	>5-12	MR (Moderate Resistant)
5	>12-25	MS (Moderate Susceptible)
7	>25-50	S (Susceptible)
9	>50-100	VS (Very Susceptible)

Evaluation of flowering behaviour which supports outcrossing ability between new CMS and their maintainer

Five new CMS lines (BI485A, BI599A, BI619A, BI639A dan BI665A) and their maintainers selected from the first experiment were evaluated for flowering behaviour. IR58025A was used as check line. The field experiment was conducted using randomized complete block design with three replicates. The days to flower, panicle exertion, stigma exertion, duration of glume opening during anthesis, angle of glume opening and seed set were observed based on SES (IRRI, 2002). Statistical analysis was conducted using SAS 9.0.

Results and Discussion

Development of cytoplasmic male sterile lines through succesive backcross

The successful use of hybrid vigor in rice depends on the availability of local cytoplasmic male sterile (CMS) and restorer lines. Cytoplasmic male sterile line is a rice genotype having abnormal anther. CMS anther has no pollen or has pollen, but easy to rupture, then there is no seed set yielded by self fertilization (Yuan *et al*., 2003). Male sterile character facilitates natural crossing thus ease the hybrid seed production.

Table 2 shows pollen sterility character of ten CMS from first backcross generation (F_1BC_1) to fifth generation (F_1BC_5). From the research, we identified six CMS candidates of Wild Abortive, three candidates of Kalinga and one candidate of Gambica types. Backcrosses formed segregating population and gave chance for breeder to select based on spesific target, in this case pollen sterility. The three cytoplasm types gave different pattern of pollen sterility in the five generations of backcrosses. In the WA type, there were unique pattern shown by BI497A and BI703A lines, in which both lines were completely sterile (100%) in the first and second generation of backcrosses, but reversed to be partial fertile on third and fourth generation. This phenomenon indicated that both lines showed unstable pollen sterility. Backcross should be done in several generations to determine pollen stability of both lines. The other lines, i.e. BI485A, BI543A, BI571A and BI599A showed positive increase in sterility percentage from first to fifth generation of backcrosses. The CMS with Gambiaca and Kalinga cytoplasm sources also increased pollen sterility as backcross progressing.

Table 2. Pollen sterility character of new cytoplasmic male sterile lines on F_1BC_1 - F_1BC_5 generation

CMS Lines	Pollen sterility on each generation (%)				
	F_1BC_1	F_1BC_2	F_1BC_3	F_1BC_4	F_1BC_5
CMS WA type:					
BI485A	98.0 ± 0.1	98.6 ± 1.8	99.8 ± 0.4	100.0 ± 0.1	100.0 ± 0.0
BI497A	100.0 ± 0.0	100.0 ± 0.0	98.4 ± 3.7	99.8 ± 0.4	100.0 ± 0.0
BI543A	94.3 ± 8.7	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
BI571A	92.0 ± 6.8	98.4 ± 2.0	98.8 ± 2.6	100.0 ± 0.0	100.0 ± 0.0
BI599A	93.8 ± 5.6	96.3 ± 5.3	97.6 ± 3.9	99.9 ± 0.2	100.0 ± 0.0
BI703A	100.0 ± 0.0	100.0 ± 0.0	99.8 ± 0.4	100.0 ± 0.1	100.0 ± 0.0
CMS Kalinga type					
BI639A	98.8 ± 2.5	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
BI665A	96.3 ± 4.2	94.3 ± 14.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
BI669A	85.3 ± 11.2	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
CMS Gambiaca type					
BI855A	95.2 ± 4.7	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0

Note: WA= *wild abortive*; value after ± was standard deviation

All completely sterile CMS' with WA cytoplasm source were achieved in the fifth generation (F_1BC_5). In the fourth backcross generation, four lines showed 100% pollen sterility (*completety sterile*), while other lines ranged from 99.8 to 99.9%. Different patterns is shown by CMS of Kalinga and Gambiaca types. The pollen stability of both CMS types were achieved earlier. All of CMS with Kalinga and Gambiaca cytoplasm had more stable pollen sterility than that of Wild Abortive. A commercially usable CMS line needs to have complete and stable male sterility, independent from environmental changes specially temperature (Yuan & Fu, 1995), adaptive to tropical rice growing conditions and possess good outcrossing potential to affect an economically viable hybrid seed production (Virmani & Kumar, 2004).

Resistance evaluation of the new cytoplamic male sterile lines to bacterial leaf blight

Male sterile lines should have good adaptation to their rice growing conditions and resistant to rice pest and disease. The three type of CMS were converted from dihaploid maintainer (DH_2) lines. The maintainers were obtained from anther culture of F_1 derived from crossing between elite maintainer lines and donor lines having high resistance to bacterial leaf blight. Both of maintainer lines and donor lines were elite breeding lines originated from Indonesia. Therefore, in the fifth backcross, the new CMS already had cytoplasm from introduced lines, but having 98.4375% nucleus genes from their male parents (*recurrent parent*). The new CMS' are expected be more adapted to Indonesian environment.

Bacterial leaf blight (BLB) caused by *Xanthomonas oryzae* pv. *oryzae* is one of the most important disease of rice which cause substantial yield losses (Lee *et al*., 2000). We tested 10 new CMS lines for reaction to three Indonesian pathotypes. The new CMS shows variation in resistance to bacterial leaf blight (Table 3). The new CMS of wild abortive cytoplasm and Kalinga cytoplasm showed to be resistant to moderate resistant to pathotype III, while Gambiaca cytoplasm was resistant to pathotype III. In Indonesia, the pathotype IV and VIII of bacterial leaf blight were more virulence than pathotype III. The results showed variation in the reaction of all CMS from three cytoplasm sources to different pathotypes. One CMS of Kalinga type (BI665A) was resistant to both of pathotypes. The BI485A, BI497A, BI599A and BI855A showed moderate resistance to bacterial leaf blight pathotype IV. Reaction of CMS to patotype VIII also varied. BI543A and 571A were resistant to the pathotype VIII, while BI703A and BI669A were moderate resistant to the pathotype VIII.

Table 3. Reaction of cytoplasmic male sterile lines to three Indonesian pathotype of bacterial leaf blight

Cytoplasmic Male Sterile Lines	Resistance to Bacterial Leaf Blight (BLB)		
	Pathotype III	Pathotype IV	Pathotype VIII
CMS WA type:			
BI485A	3	3	5
BI497A	3	3	5
BI543A	3	7	1
BI571A	3	5	1
BI599A	3	3	5
BI703A	1	5	3
CMS Kalinga type:			
BI639A	3	7	5
BI665A	3	1	1
BI669A	1	5	3
CMS Gambiaca type:			
BI855A	1	3	5

Note: 1 Resistant, 3 Moderate Resistant, 5 Moderate Susceptible, 7 Susceptible, 9 Very Susceptible

Sudir & Suprihanto (2006) reported that virulence of the bacterial *Xanthomonas oryzae* pv. *oryzae* (Xoo) was significant in affecting disease severity of BLB. The elite line donor having *xa5* gene which has responsibility to disease resistance. The *xa5* gene is recessive and constitutively expressed at the same level as the dominant susceptible allele, *Xa5*, and neither allele is induced or suppresed in response to Xoo (Bart *et al*., 2006). Availability of CMS lines stable in sterility, good in agronomic characters, and resistant to disease will increase the adaptability of the hybrid developed from those CMS lines.

Evaluation of flowering behaviour that support the outcrossing ability between new CMS and their maintainer

Table 4 shows the observation of several flower behavior of five CMS lines. IR58025A was used as check line because most rice hybrids in Indonesia were derived from this line. New CMS lines were earlier to flower than IR58025A, except BI665A. The maintainers flowered 1–4 days before their respective CMS pairs. The different days to flower between CMS and their respective maintainers were in the threshold and important for flowering syncronization when produced CMS seed (Virmani *et al.* 1997).

Table 4. Flowering behavior of five new CMS and their maintainers

Cytoplasmic Male Sterile Lines	DF (days)		PE (%)	GOD (minutes)		GOA (°)		SE (%)	Seed Set (%)	
	A	B	A	A	B	A	B	A	A	B
CMS *Wild Abortive type* :										
BI 485	76.7 d	75.0 c	77.1 b	143.9 ab	38.4 b	34.9 bc	40.3 a	74.7 a	19.4 ab	90.8 a
BI 599	79.7 c	75.0 c	80.0 ab	162.9 a	52.1 a	39.8 a	35.6 b	71.8 ab	4.8 c	74.4 c
CMS Gambiaca type:										
BI 855	81.7 c	77.0 c	82.1 a	132.5 bc	28.6 b	36.0 b	36.5 ab	75.7 a	10.8 bc	91.5 a
CMS Kalinga type:										
BI 639	81.0 c	76.0 c	80.9 a	120.6 c	54.3 a	40.6 a	35.9 b	67.7 b	25.9 a	89.4 ab
BI 665	91.0 a	91.7 a	75.5 c	135.9 bc	41.9 ab	33.7 bc	38.3 ab	73.4 ab	15.7 b	65.2 d
Check (WA):										
IR58025A	88.0 b	88.3 b	81.0 a	121.8 c	54.3 a	32.8 c	29.3 c	43.3 c	2.9 c	81.7 ab
CV (%)	1.7	1.4	2.0	8.5	15.6	3.5	5.8	5.2	18.5	5.7

Note: A: cytoplasmic male sterile lines, B: maintainers; PE: panicle exsertion, SE: stigma exsertion, GOD: duration of glume opening during anthesis, GOA: angle of glume opening during anthesis; seed set formed in CMS (A) was yielded by natural outcrossing, pollen came from their respective maintainers; seed set of each maintainer (B) was obtained by selfing

All new CMS' showed good panicle exsertion (more than75%), but BI485A (WA) and BI665 (Kalinga) were more enclosed than IR58025A. Duration of glume opening during anthesis of BI485A and BI599A (WA type) was significantly longer than IR58025A. The other new CMS lines also showed longer duration of glume opening than that of IR58025A but not statistically significant. BI599A, BI855A and BI639A showed wider angle of glume opening than that of IR58025A i.e. 39.8; 36.0 and 40.6 degrees, respectively. The better duration and angle of glume opening would increase the opportunity of new CMS stigmas to receive pollen from maintainers.

Stigma exsertion of the three types of CMS was higher than IR58025A. It ranged from 67.7 to 74.7% compared to that of IR58025A (43.3%). The better flowering behavior caused higher seed set of the new CMS than IR58025A. The new lines set seed at about 4.8–25.9%, while IR58025A achieved 2.9% of seed set. Corellation analysis showed positive high and significant value between seed set of new CMS lines with stigma width (r=0.44*), stigma exertion (r=0.54*) and angle of glume opening during anthesis (r=0.42*). The seed set was also affected by some flower characters of maintainer lines such as filament length and angle of glume opening during anthesis (data not shown). The results indicated that the new CMS lines with different cytoplasm source could be used in hybrid rice breeding.

Conclusions

Combination of anther culture and backcrosses obtained 6 CMS lines of WA type, 3 CMS lines of Kalinga type, and 1 CMS line of Gambiaca type. The lines had stable sterility and resistance to 1-3 *Xanthomonas oryzae* pathotypes. The new CMS showed better flowering behavior which increase the seed set.

References

Bart, R., P. Ronald, and S. Hake. 2006. Fertility versus disease resistance, a hard choice. Gene and Development (20): 1215-1217.

Dewi, I.S., A. Apriana, A. Sisharmini, and I.H. Somantri. 2007. Resistance evaluation of doubled haploid of parental lines candidate to bacterial leaf blight and brown planthopper. Bull. Agron 35(1):15-21.

Eckardt, N.A. 2006. Cytoplasmic male sterility and fertility restoration. The Plant Cell 18:515-517.

Gavino, R.B., Y. Pi, and C.C. Abon Jr. 2008. Application of gibberellic acid (GA3) in dosage for three hybrid rice seed production in the Philippines. Journal of Agricultural Technology 4(1): 183-192.

[IRRI] International Rice Research Institute. 2002. Standarization Evaluation System for Rice. 66p

Rumanti I.A., Dewi I.S., Purwoko B.S. and Aswidinnoor H. 2008. Evaluation of anther culture-derived doubled haploid maintainer lines for developing rice male sterile lines. Jurnal Agronomy Indonesia 37 (1): 1 – 7 (In Indonesian).

Takai, T., S. Matsura, T. Nishio, A. Ohsumi, T. Shiraiwa, and T. Horie. 2006. Rice yield potential is closely related to crop growth rate during late reproductive periode. Field Crops Research 96: 328-335.

Li, J., S. Cai, J. Feng, Li, and G. Cheng. 2007. Comparisons on genetic diversity among the isonuclear-allopasmic male sterile lines and their maintainer lines in rice. Rice Science 14(2): 94-100.

Peng, S., G.S. Khush, P. Virk, Q. Tang, and Y. Zou. 2008. Progress in ideotype breeding in increase rice yield potential. Field Crop Research 108: 32-38.

Sudir and Suprihanto. 2006. Change in virulence of strain *Xanthomonas oryzae* pv. *oryzae*, a causal pathogen of bacterial leaf blight in rice. Jurnal Penelitian Pertanian Tanaman Pangan 25 (2): 100-107. (In Indonesian).

Suwarno, N.W. Nuswantoro, Y.P. Munarso, and M. Diredja. 2003. Hybrid rice research in Indonesia..In: S.S. Virmani, C.X. Mao, and B. Hardy, eds, Hybrid Rice for Food Security, Poverty Alleviation, and Environmental Protection. Proceedings of the 4th International Symposium on Hybrid Rice, Hanoi-Vietnam, 14-17 May 2002. IRRI, Philippines. pp. 287-296.

Virmani, S.S., and I. Kumar. 2004. Development and use of hybrid rice technology to increase rice productivity in the tropics. Internationasl Rice Research Notes 29 (1):10-19.

Virmani, S.S., B.C. Viraktamath, C.L. Casal, R.S. Toledo, M.T. Lopez, and J.O. Manalo. 1998. Hybrid Rice Breeding Manual. IRRI, Philippines.

You, A., X. Lu, H. Jin, X. Ren, K. Liu, G. Yang, H. Yang, L. Zhu, and G. He. 2006. Identification of quantitative trait loci across recombinant inbred lines and testcross populations for traits of agronomic importance in rice. Genetics 172:1287-1300.

Yuan, L.P., X. Wu, L. Fuming, G. Ma, and Q. Xu. 2003. Hybrid Rice Technology. China Agriculture Press.

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