Kasetsart J. (Nat. Sci.) 47 : 675 - 683 (2013)

Development of A, B and R Lines by Gamma Irradiation for Hybrid Rice

Anucha Mekaroon[1], Choosak Jompuk[1], Rungsarid Kaveeta[2], Bang-orn Thammasamisorn[3] and Peeranuch Jompuk[4,*]

ABSTRACT

The development of hybrid rice holds great potential for increasing yields, but there are limitations on how hybrid rice seed can be produced. Exploiting rice male sterility genes is one way to facilitate hybrid seed production, but high yield and vigor in a hybrid depend on heterosis resulting from the genetic diversity of the male and female parents. The objective of this research was to create greater genetic diversity in male sterile, or A lines, maintainer lines, or B lines, and restorer lines, or R lines, for hybrid seed production by inducing mutations using gamma radiation. New A and R lines were created by exposing CP304 hybrid rice to acute gamma irradiation at doses of 0, 100, 200 and 300 Gy. New B lines were developed by taking the F_1 generation of crosses between three B lines (IR68886B, IR68888B and IR68899B) and an R line (SPR90119R) and exposing the plants at the booting stage to chronic gamma irradiation at a dose of 416 Gy at the Nuclear Technology Research Center, Kasetsart University. Then they were bred using conventional breeding. Ten A lines and three R lines (RM_3) were selected from the irradiated CP304 rice. Ten B lines were selected from the M_3 generation of B × R line crosses. Ten counterpart A and B lines were developed by backcrossing to obtain the A(BC_1) and BM_3 generations. F_1 hybrid rice was formed by crossing A(BC_1) × RM_4 using the line × tester method for 30 crosses. Yield trials revealed that the hybrids gave yields of between 0.89 and 4.73 t.ha^{-1} with a mean of 2.64 t.ha^{-1}. The highest yielding hybrid was A(BC_1)-1 × RM_4-1 with a yield of 4.73 t.ha^{-1}. The restorer line RM_4-1 had the highest general combining ability. Compared to a standard hybrid rice variety (PTT06001H) with a mean yield of 7.27 t.ha^{-1}, the yield of the hybrids produced in this research was still low because the three R lines were heterozygous (Rr) for the fertility restoration gene and the 30 hybrids segregated into male sterile and fertile plants at a ratio of 1:1. If the R lines can be further developed to be homozygous dominant (RR) then higher yielding hybrids could be achieved.

Keywords: A line, B line, R line, gamma radiation, hybrid rice

1 Department of Agronomy, Faculty of Agriculture at Kamphaeng Saen, Kasetsart University, Nakhon Pathom 73140, Thailand.

2 Department of Agronomy, Faculty of Agriculture, Kasetsart University, Bangkok 10900,Thailand.

3 Suphan Buri Rice Research Center, Bureau of Rice Research and Development, Rice Department, Bangkok Thailand.

4 Department of Applied Radiation and Isotopes, Faculty of Science, Kasetsart University, Bangkok 10900, Thailand.

* Corresponding author, e-mail: fsciprk@ku.ac.th

Received date : 30/04/13 Accepted date : 16/07/13

INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most important cereal crops in the world because it provides food for more than two billion people (Lampe, 1995). According to Office of Agricultural Economics (2012), in Thailand, rice is the staple food and the country's main economic crop; in 2011 there were 9.76 million ha of land devoted to planting rice for the main (rain-fed) season and 2.72 million ha of land used for growing a second (irrigated) crop of rice. Furthermore, the total crop in 2011 amounted to 33 million t and the export value of rice and rice products was 210.527 billion baht (approximately USD 6.7 billion). Nevertheless, Thailand's average rice productivity is quite low at only 2.38 $t.ha^{-1}$ for the rainy season crop and 4.14 $t.ha^{-1}$ for the second crop. However, Thailand is still one of the top rice exporting countries of the world, but Thailand's productivity is low compared to its competitors (Office of Agricultural Economics, 2012). Besides conventional efforts to improve rice varieties, the development of hybrid rice is another promising way of increasing yield. In China, hybrid rice was shown to give 15–20% higher yield than ordinary pure lines in a farmer's field. The development of hybrid rice is facilitated by the use of a male sterile line. The first set of cytoplasmic male sterility (CMS, or three-line) systems was produced in 1970, while the first hybrid rice was released in 1974, with the hybrids outyielding, on average, the conventional rice varieties by 20% (Guohui and Longping, 2003). The three-line system requires a cytoplasmic male sterile (A line), a maintainer (B line) and a restorer (R line) to produce F_1 seeds (Jiming *et al*., 2009). Hybrid rice has an advantage not only in yield but also in combining resistance to diseases and pests found in the parents. Moreover, hybrid rice possesses another important characteristic, adaptability to various environmental constraints, especially drought (Virmani, 1996). In Thailand, work to develop hybrid rice has been proceeding for some time now. While the Rice Department has released a hybrid rice variety (PTT06001H) for commercial trials and a private company is also selling a hybrid rice variety called CP304, the yield of these new hybrid rice varieties is still not very high (Biothai Foundation, 2012). This may be partly because of a lack of genetic diversity among the lines that have been crossed to develop them. The A and B lines are still being improved in order to make them better adapted to the climate and growing conditions in Thailand, because the male sterile germplasm was obtained from the International Rice Research Institute (IRRI) (Amornsilpa, 1996). Also, more work needs to be done to identify restorer lines (R line) that can contribute to heterosis and greater yield when crossed with the male sterile lines. The success of a hybrid is dependent on the level of heterosis that can be expressed after crossing the parental varieties. The combination between different varieties is the first step to obtain heterosis, but its expression improves as combinations between varieties belonging to different groups (*indica* and *japonica*) are explored (Guimaraes, 2009). An alternative that can be used to develop hybrids with higher potential might be the use of yield-enhancing genes from other species (Longping, 2003). Molecular markers are being used to try to identify restorer genes in the background of *japonica* (Tan *et al*., 1998) and marker-assisted selection has been reported to assist in the development of hybrids with disease and insect resistance in China (He *et al*., 2004). The use of different sources derived from induced mutations was a popular choice to generate genetic diversity for specific traits in rice in the 1980s and today the technique has become part of the toolkit breeders use to enhance specific rice characteristics in well-adapted varieties, with the most popular mutagen still being gamma rays (Guimaraes, 2009). However, commercial hybrid rice varieties can be used as a source of genetic material to produce better A lines and R lines. Also,

mutation induction through gamma irradiation is one way to create greater genetic diversity among male sterile lines before backcrossing them and testing the general combining ability of the F_1 generation. The objective of this research was to produce a superior A line and R lines for hybrid rice production by inducing mutations in commercial hybrid rice varieties via gamma irradiation and to produce an improved B line by crossing B × R lines in combination with gamma irradiation.

MATERIALS AND METHODS

Gamma ray mutation induction to develop A and R lines

Seeds of hybrid rice variety CP304 were exposed to acute gamma irradiation at doses of 0, 100, 200 and 300 Gy to obtain M_1 seeds.

Season 1 November 2009–March 2010

M_1 seeds that had been exposed to 0, 100, 200, or 300 Gy gamma radiation were sprouted and transplanted singly (one plant per hole), when the seedlings were aged 21 d at the experimental field of the Agronomy Department, Faculty of Agriculture at Kamphaeng Saen, Kasetsart University, Kamphaeng Saen campus, Nakhon Pathom. The numbers of transplanted plants were 120 for the 0 Gy group, 1,600 for the 100 Gy group, 1,600 for the 200 Gy group and 1,040 for the 300 Gy group. The seeds of each plant were harvested separately.

Season 2 August 2010–November 2010

M_2 seeds were collected from 202 M_1 plants from the 100 Gy group, 204 from the 200 Gy group and 118 from the 300 Gy group. From these M_2 seeds, a total of 4,534, 4,305 and 2,397 plants were obtained, respectively. In theory, when hybrid rice is selfed once, the progeny will segregate into male sterile (AM_2) and restorer lines (RM_2) (Virmani and Sharma,1993). In this experiment, the restorer lines were selected and selfed again to obtain the RM_3 generation, while the male sterile lines (AM_2) were reproduced by node propagation.

Season 3 February 2011–May 2011

The RM_3 generation yielded 480 lines, which were planted out line-to-row and selfed to obtain the RM_4 generation. From these, 16 high-yielding restorer lines with good agronomical traits were selected.

Gamma ray mutation induction to develop B lines

Three B lines were crossed with one R line in the following combinations to produce the F_1 hybrid generation: IR68886B × SPR90119R, IR68888B × SPR90119R and IR68899B × SPR90119R. F_1 hybrid seeds were planted in pots and when they reached the booting stage they were exposed to chronic gamma irradiation at the rate of 1.07 $Gy.hr^{-1}$, spaced 1.5 m from the radiation source, for a dosage of 416 Gy. The seeds from the irradiated plants were collected as M_1 seeds from each cross.

Season 1 November 2009–March 2010

The irradiated M_1 seeds were planted in pots and selfed to obtain M_2 seeds from each of the three crosses. The seeds from each cross were combined in bulk.

Season 2 August 2010–November 2010

Three hundred M_2 seeds from each cross were grown in the experimental field (25 × 25 cm spacing) and selfed. Seeds from the individuals with the best agricultural traits (good tillering, longest grains and highest percentage of seed set) were selected for the M_3 generation.

Season 3 February 2011–May 2011

The M_3 seeds, which could segregate into B lines and R lines, were sprouted and then

transplanted (25 × 25 cm spacing) at the rate of 168 plants per line. At the same time, 94 male sterile lines (A line; AM_2) that came from node propagation of irradiated CP304 hybrid rice were planted. The five best M_3 individuals obtained from the three crosses were then randomly crossed with the male sterile lines (AM_2).

Season 4 May 2011–August 2011

To determine which of the selected M_3 lines were B lines or R lines, the F_1 seeds of the 34 AM_2 × M_3 (all 3 pairs) crosses were planted. If any F_1 plant was found to have sterile pollen, it signified that the M_3 parent was a B line. If any F_1 plant had normal pollen, it meant that the M_3 parent was an R line. The F_1 hybrids with sterile pollen would be developed as A lines and complementary B lines would be obtained by crossing with the corresponding BM_4 line. The M_3 plants that were identified as R lines were left to self to form the RM_4 generation.

Season 5 September 2011–December 2011

Ten A lines ($A(BC_1)$) were selected consisting of: five from the AM_2 × (IR68886B × SPR90119R)-BM_4 cross, one from the AM_2 × (IR68888B × SPR90119R)-BM_4 cross and four from the AM_2 × (IR68899B × SPR90119R)-BM_4 cross. The ten $A(BC_1)$ male sterile lines were planted along with their counterpart BM_5 lines and backcrossed to increase the genetic similarity between the A line and B line. Thus the $A(BC_2)$ generation was obtained.

Hybridization and yield testing

Season 5 September 2011–December 2011

Line × tester hybrids were created by crossing the 10 $A(BC_1)$ lines with the three RM_4 restorer lines (CP304-M_4-70-2-1-1, CP304-M_4-14-2-1-1 and CP304-M_4-36-2-1-1) for a total of 30 F_1 hybrids.

Season 6 February 2012–May 2012

To determine which of the A lines had the best combining ability for crossing with the R lines, the F_1 seeds from the 30 line × tester hybrid crosses were then grown and field tested together with five standard rice varieties for comparison (Pinkaset, Suphanburi 31, Pathum Thani 1, hybrid CP304 and hybrid PTT06001H). They were planted in rows 4 m apart with three rows of each cross in a randomized complete block design in four replications.

RESULTS AND DISCUSSION

Development of male-sterile lines (A lines) and restorer lines (R lines)

When seeds of hybrid rice CP304 were exposed to acute gamma radiation at doses of 0, 100, 200, and 300 Gy, the survival rates were 100, 73.1, 61.9 and 43.0%, respectively. There was a statistically significant ($P < 0.05$) negative relationship between the survival rate and the radiation dose. This was consistent with the findings of previous research, although the response to gamma radiation varies with different kinds of plants (Wongpiyasatid, 1989). The current results showed that the 50% lethal dose ($LD_{50(30)}$; the amount of radiation that caused half the CP304 rice seeds to die compared to the non-irradiated control after 30 d) was 280 Gy. Some of the irradiated seeds (M_1) displayed abnormal growth when planted out. This may have been due to damage caused by the radiation, which may have produced a permanent mutation or may simply have been transitory physiological damage (Lamseejan *et al.*, 1988). The International Atomic Energy Agency (1977) reported that the most effective dosage range for inducing mutations through acute radiation treatment in *indica* rice was 110–300 Gy. Thus, for the current research, the doses of 100, 200, and 300 Gy were chosen.

For the first mutated generation (M_1), the irradiated rice seeds were planted in seedling trays and then transplanted (25 × 25 cm spacing) when they were aged 21 d. The number of plants from each radiation dose treatment was different. There were 120, 1,600, 1,600 and 1,040 M_1 plants from the treatments of 0, 100, 200 and 300 Gy, respectively (Table 1). There was no segregation of the male sterile trait in the M_1 generation. At harvest time, seeds were collected from 1,184 clumps from the 100 Gy group, 663 clumps from the 200 Gy group and 512 clumps from the 300 Gy group. The harvested seeds were designated the M_2 seeds. The next season, these M_2 seeds were sprouted (the M_2 plants) and were transplanted at age 21 d to select the male sterile (AM_2) and restorer (RM_2) lines. Of the 11,236 M_2 plants 2,807 were male sterile and did not set seed, while the other 8,429 were male fertile (Table 1). Out of these, 119 of the male sterile plants and 647 of the male fertile plants were selected for having good agronomic traits. The segregation ratio of male sterile to male fertile plants was not the expected 3:1 except for the 200 Gy treatment group. Normally, for rice that is not radiated, after the hybrid F_1 progenies are selfed to obtain the F_2 generation, the ratio of male fertile to male sterile plants will be 3:1 because the gene for restoring fertility (RR) will be in the heterozygous state (Rr) while the gene for sterility (S) is present in the cytoplasm. For the second generation of non-irradiated hybrid plants (M_2) the expected segregation rate should be one part RR to two parts Rr (all of which will have the fertile phenotype) to one part rr (which will be completely sterile), and all of them will have the cytoplasmic male sterility gene S in their cytoplasmic DNA (Virmani *et al.*,1997).

One reason the segregation ratio differed from 3:1 in the current experiment may have been because of the radiation. Gamma radiation can cause genetic changes in plants at the individual gene level and at the chromosome level. Mutations at the chromosome level may be lethal to plants or may cause sterility when the number of chromosomes is uneven (Lamseejan *et al*., 2003). The aim of this experiment was to induce other kinds of genetic changes, because CP304 hybrid rice already contains genes for male sterility. The goal was to select new male-sterile lines for use in the same 3-line hybridization system with different traits from the original CP304 hybrid variety. For this generation, the male sterile lines could be maintained via nodal propagation while new restorer lines could be selected from the male fertile offspring with good agronomic traits and good seed set. The selected plants were selfed to obtain the RM_3 seed. The RM_3 lines could have either the RR or Rr genotype, but only those with the RR genotype are useful for producing hybrid

Table 1 Number of planted, harvested and selected (M_1 and M_2) lines of commercial hybrid rice variety CP304 treated with different gamma radiation doses.

Dose (Gy)	No. of M_1 plants		No. of M_2 plants					Selected M_2 plants	
			Grown out						
	Grown	Harvested	Plants	Male fertile	Male sterile	χ^2 (3:1)[a]	Albino	Male fertile	Male sterile
100	1,600	1,184	4,534	3,475	1,059	*	26	206	38
200	1,600	663	4,305	3,259	1,046	ns	21	310	41
300	1,040	512	2,397	1,695	702	**	7	158	40
Total	4,360	2,359	11,236	6,429	2,807	**	54	674	119

[a] = Chi-square test between male fertile and male sterile M_2 plants

ns = Not significant ($P > 0.05$), * = Significant ($P \leq 0.05$), ** = Highly significant ($P \leq 0.01$).

rice seed. Thus, it is necessary to cross the RM_3 plants with male sterile (A line) plants in order to test the F_1 generation to identify if the progeny have fertile pollen. If all the progeny of a given RM_3 line have fertile pollen, then it shows that that line has the RR genotype; but if some of the F_1 plants have fertile pollen and others are sterile, then it shows that that RM_3 line has the Rr genotype (Virmani *et al.*,1997).

For this research, 480 RM_3 restorer lines were planted and the 16 lines that showed the best agronomic traits and highest yield were selected. They were selfed to obtain the fourth generation RM_4 and in the next season the three best (RM_4) R lines were selected and designated CP304-M_4-70-2-1-1, CP304-M_4-14-2-1-1 and CP304-M_4-36-2-1-1 from the doses of 100, 200 and 300 Gy, respectively, to be used as testers. Nevertheless, these selected testers might have either RR or Rr genotypes. To maintain the male sterile line (AM_2), 10 male sterile lines from each dose were propagated by nodal cutting and were used as the A line in the experiment.

Development of maintainer lines (B lines) *via* gamma radiation mutation

Booting-stage rice plants from the first generation (F_1) hybrids of the three crosses of B lines with an R line (IR68886B × SPR90119R, IR68888B × SPR90119R and IR68899B × SPR90119R) were exposed to a chronic gamma radiation dose of 416 Gy. The radiated (M_1) seeds were then grown and selfed for two seasons (M_2 and M_3) to select those with the best agronomical characteristics (good tillering, long grain seeds and good seed set). For the M_2 generation, 30 lines were selected from the (IR68886B × SPR90119R)-M_2 cross, 12 from the (IR68888B × SPR90119R)-M_2 cross and 28 from the (IR68899B × SPR90119R)-M_2 cross (Table 2). They were selfed to obtain the M_3 seeds. In theory, the second-generation progeny of (F_2) of a cross between a maintainer line and a restorer line should segregate into B lines and R lines in a 1:1 ratio (Virmani and Sharma, 1993). This research selected five M_3 lines from each of the three crosses and crossed them randomly (random mating) with the male sterile lines (AM_2) that were obtained from irradiating CP304 hybrid rice and growing the sterile plants via node propagation. Seeds of 27 A lines were obtained, consisting of 11 from the AM_2 × (IR68886B × SPR90119R)-M_3 cross, 5 from the AM_2 × (IR68888B × SPR90119R)-M_3 cross, and 11 from the AM_2 × (IR68899B × SPR90119R)-M_3 cross. In addition, M_4 seeds were collected from the 15 selected M_3 lines. The 27 hybrid crosses were grown out to test the fertility restoration ability of the M_3 and the results showed that the hybrids from 14 of the crosses had normal pollen and 13 had sterile pollen, consisting of 6 from the AM_2 × (IR68886B × SPR90119R)-M_3 cross, 2 from the AM_2 ×

Table 2 Number of B and R lines from the segregation of M_3 plants from the crosses of IR68886B × SPR90119R, IR68888B × SPR90119R and IR68899B × SPR90119R.

Cross (M_1)	M_2 selected	No. of Cross $AM_2 \times M_3$	M_3			A(BC_1) and BM_5
			B line	R line	χ^2 test(1:1)[a]	
IR68886B × SPR90119R	30	11	6	5	ns	5
IR68888B × SPR90119R	12	5	2	3	ns	1
IR68899B × SPR90119R	28	11	5	6	ns	4
Total	70	27	13	14	ns	10

[a] = Chi-square test between B and R lines of M_3.
ns = Not significant (P > 0.05).

(IR68888B × SPR90119R)-M_3 cross and 5 from the AM_2 × (IR68899B × SPR90119R)-M_3 cross. Normally, when a male sterile line is crossed with a tester line that contains the R gene, the progeny may be either sterile or normal; if they are sterile, then it shows that the tester line is a maintainer or B line; if the progeny have fertile pollen, then it shows that the tester line is a restorer line or R line (Virmani and Sharma, 1993). In order to maintain the male sterile lines that were selected, pollen was taken from the counterpart M_3 lines that had been identified as B lines and had selfed to form M_4 seed, or what was then designated BM_4 seed. When crossed with their counterparts, this resulted in the first hybrid backcross generation or $A(BC_1)$. The BM_4 planted were selfed to obtain the BM_5 seeds. Finally, 10 pairs of male sterile lines ($A(BC_1)$) with corresponding maintainer lines (BM_5) were obtained, consisting of 5 from the cross AM_2 × (IR68886B × SPR90119R)-BM_4, 1 from the cross AM_2 × (IR68888B × SPR90119R)-BM_4 and 4 from the cross AM_2 × (IR68899B × SPR90119R)-BM_4. For the $A(BC_1)$ line, the percentage of male sterility ranged from 98 to 100%. The average of plant height, tillering and panicle were approximately 79 cm, 35 tillers per plant, and 22 panicles per plant, respectively.

Hybrid yield trial

Line × tester hybrids were generated by crossing the 10 $A(BC_1)$ lines with three restorer (RM_4) lines, resulting in F_1 seed from 30 crosses of parent plants, which were planted out to test their field performance. The average yield of the hybrids was 2.64 t.ha^{-1} with a range from 0.90 to 4.73 t.ha^{-1} (Table 3). The hybrids with the five highest yields were $A(BC_1)$-1× RM_4-1 (4.73 t.ha^{-1}), $A(BC_1)$-6 × RM_4-1 (4.65 t.ha^{-1}), $A(BC_1)$-5 × RM_4-1 (4.46 t.ha^{-1}), $A(BC_1)$-3 × RM_4-3 (4.14 t.ha^{-1}) and $A(BC_1)$-7 × RM_4-3 (3.56 t.ha^{-1}). The restorer line RM_4-1 resulted in a good mean yield (3.39 t.ha^{-1}) when crossed with all 10 male

Table 3 Mean grain yield, general combining ability (GCA) and specific combining ability (SCA) of Line × Tester mating design.

Line	Yield (t.ha^{-1}) and Combining ability in parenthesis							
	Tester						Mean (GCA line)	
	RM_4-1		RM_4-2		RM_4-3			
$A(BC_1)$-1	4.73	(0.90)**	3.32	(0.80)**	1.20	(-1.69)**	3.08	(0.44)**
$A(BC_1)$-2	3.55	(0.08)^{ns}	2.22	(0.06)^{ns}	2.39	(-0.14)^{ns}	2.72	(0.08) ^{ns}
$A(BC_1)$-3	2.66	(-1.23)**	2.63	(0.05)^{ns}	4.14	(1.18)**	3.14	(0.50)**
$A(BC_1)$-4	3.44	(0.37)*	2.25	(0.49)**	1.28	(-0.86)**	2.32	(-0.32)**
$A(BC_1)$-5	4.46	(0.30) ^{ns}	2.62	(-0.23)^{ns}	3.16	(-0.07)^{ns}	3.42	(0.77)**
$A(BC_1)$-6	4.65	(0.78)**	1.96	(-0.59)**	2.73	(-0.19)^{ns}	3.11	(0.47)**
$A(BC_1)$-7	3.06	(-0.19)^{ns}	0.90	(-1.05)**	3.56	(1.24)**	2.50	(-0.14)^{ns}
$A(BC_1)$-8	2.23	(-0.78)**	2.00	(0.30)^{ns}	2.55	(0.48)**	2.26	(-0.38)**
$A(BC_1)$-9	1.81	(-0.53)**	1.16	(0.13)^{ns}	1.81	(0.41)*	1.59	(-1.05)**
$A(BC_1)$-10	3.33	(0.31)*	1.75	(0.05)^{ns}	1.72	(-0.36)*	2.27	(-0.38)**
Mean (GCA tester)	3.39	(0.75)**	2.08	(-0.56)**	2.45	(-0.19)*	2.64	

ns = Not significant ($P > 0.05$), * = Significant difference ($P < 0.05$), ** = Highly significant difference ($P < 0.01$), $LSD_{(0.05)}$ = 0.45, $LSD_{(0.01)}$ = 0.65.

Check variety: Pinkaset1 = 2.18 t.ha^{-1}, Suphanburi 31 = 6.34 t.ha^{-1}, Pathumthani 1=7.61 t.ha^{-1}, CP 304 = 3.01 t.ha^{-1}and PTT06001H = 7.28 t.ha^{-1}.

Standard error for combining ability: SE (GCA for line) = 0.11, SE (GCA for tester) = 0.06, SE (SCA effect) = 0.19.

sterile lines, while the RM_4-2 and R5 lines produced lower yields, both at about the same average (means of 2.08 and 2.45 $t.ha^{-1}$, respectively). Tests of general combining ability (GCA) revealed that of the R lines (RM_4-1) had the highest GCA. For A lines, the GCA of the $A(BC_1)$-5, $A(BC_1)$-3, $A(BC_1)$-6 and $A(BC_1)$-1 lines were rated as good. When yield was considered together with specific combining ability (SCA), the $A(BC_1)$-1, $A(BC_1)$-5, $A(BC_1)$-6 lines and the RM_4-1 restorer line were considered promising. Of secondary interest were the $A(BC_1)$-3 and $A(BC_1)$-7 lines, which had high SCA with the RM_4-3 line and yielded 4.11 and 3.56 $t.ha^{-1}$, respectively. In conclusion, there were 2 groups of A lines and R lines that can be used to develop hybrid rice varieties in the future: Group 1, consisting of $A(BC_1)$-1, $A(BC_1)$-5 and $A(BC_1)$-6 with the restorer RM_4-1; and Group 2, consisting of $A(BC_1)$-3 and $A(BC_1)$-7 with the restorer RM_4-3.

However, when compared to common commercial rice varieties, the hybrids developed in this experiment had rather low yields, with a maximum of 4.73 $t.ha^{-1}$ compared to 7.61 $t.ha^{-1}$ for Pathumthani 1 and 7.28 $t.ha^{-1}$ for the hybrid rice PTT06001H. The main reason that the hybrid rice in this research had lower yields was probably because all three R lines were heterozygous for the nuclear restorer gene (Rr), resulting in F_1 hybrid rice that was either sterile or fertile plants in the ratio 1:1 (data not shown).

CONCLUSION

F_1 hybrid rice was formed by crossing $A(BC_1) \times RM_4$ using the line × tester method for 30 crosses. Yield trials revealed that the hybrids gave yields of between 0.90 and 4.73 $t.ha^{-1}$ with a mean of 2.64 $t.ha^{-1}$. The highest yielding hybrid was $A(BC_1)$-1 × RM_4-1 with a yield of 4.73 $t.ha^{-1}$. The restorer line RM_4-1 had the highest general combining ability. Compared to a standard hybrid rice variety (PTT06001H) with mean yield of 7.28 $t.ha^{-1}$, the yield of the hybrids produced in this research was still low because the three R lines were heterozygous (Rr) for the fertility restoration gene. If the R lines can be further developed to be homozygous dominant (RR) then higher yielding hybrids could be achieved.

ACKNOWLEDGEMENTS

This research was financially supported by the Kasetsart University Research and Development Institute (KURDI), Kasetsart University, Bangkok, Thailand.

LITERATURE CITED

Amornsilpa, S. 1998. Hybrid rice in Thailand, pp. 409–412. *In* S.S. Virnani, E.A. Siddiq and K. Muarlidharn, (eds.). **Proceedings of the 3rd International Symposium on Hybrid Rice.** 14–16 November 1996, Hyderabad, India and International Rice Research Institute. Manila, The Philippines. 443 pp.

Biothai Foundation. 2012. The problem analysis report of hybrid rice: A case study of hybrid rice from the Charoen Pokphand Group (CP). Bangkok, Thailand. 21 pp. [Available from: http://www.biothai.net/node/150]. [Sourced: 20 April 2013].

Guimaraes, E.P. 2009. Rice breeding, pp. 99–126. *In* M.J. Carena, (ed.). **Cereals.** Springer. New York, NY, USA.

Guohui, M. and Y. Longping. 2003. Hybrid rice achievements and development in China, pp. 247-256. *In* S.S. Virmani, C.X. Mao and B. Hardy, (eds.). **Hybrid Rice for Food Security, Poverty Alleviation and Environmental Protection.** International Rice Research Institute. Manila, Philippines.

He, Y., X. Li, J. Zhang, G. Jiang, S. Liu, S. Chen, J. Tu, C. Xu and Q. Zhang. 2004. Gene pyramiding to improve hybrid rice by molecular marker techniques. *In* B.

Clements *et al.*, (eds.). **Proceedings of the 4th International Crop Science Congress.** 26 September–1 October 2004. Brisbane, Qld, Australia. [Available from: http://www.cropscience.org.au/icsc2004/]. [Sourced: 9 April 2013].

International Atomic Energy Agency. 1977. **Manual on Mutation Breeding. Technical Report Series No. 119.** 2nd ed. Joint FAO/IAEA Division of Atomic Energy in Food and Agriculture. Vienna, Austria. 288 pp.

Jiming, L., X. Yeyun and Y. Longping. 2009. **Hybrid Rice Technology Development: Ensuring China's Food Security.** International Food Policy Research Institute, Consultative Group on International Agricultural Research (CGIAR). New Delhi, India. 28 pp.

Lampe, K. 1995. Rice research: Food for 4 billion people. **Geo Journal** 35(3): 253–259.

Lamseejan, S., P. Jompuk and S. Deeseepan. 2003. Improvement of Chrysanthemum var. 'Taihie' through in vitro induced mutation with chronic and acute gamma rays. **Journal of the Nuclear Science of Thailand** 4**:** 1–4.

Lamseejan, S., S. Smutkupt. A. Wongpiyasatid and K. Naritoom. 1988. Use of radiation in mungbean breeding, pp. 174–177. *In* Anonymous (ed.). **Proceeding of the 2nd International Symposium (AVRDC Publication No. 88-304).** 16–20 November 1987. Asian Vegetable Research and Development Center. Bangkok, Thailand.

Longping, Y. 2003. A preliminary report on the male sterile in rice in Chinese with English summary. **Science Bull.** 4: 32–34.

Office of Agricultural Economics. 2012. **Agricultural Statistics of Thailand in Crop Year 2011/12.** Ministry of Agriculture and Cooperatives. Bangkok, Thailand.

Tan. X.L., A. Vanavichit, S. Amornsilpa and S. Trangoorung. 1998. Genetic analysis of rice CMS-WA fertility restoration based on QTL mapping. **Theor. Appl. Genet.** 97: 994–999.

Virmani, S.S. 1996. Hybrid rice. **Adv. Agron.** 57: 328–462.

Virmani, S.S. and H.L. Sharma. 1993. **Manual for Hybrid Rice Seed Production.** International Rice Research Institute. Manila, Philippines. 57 pp.

Virmani. S.S., B.C. Viraktamath, C.L. Casal, R.S. Toledo, M.T. Lopez and J.O. Manalo. 1997. **Hybrid Rice Breeding Manual**. International Rice Research Institute. Manila, Philippines. 151 pp.

Wongpiyasatid, A. 1989. Radiosensitivity of cotton. **Mutation Breeding Newsletter** 33: 20.