2. China

FNCA Mutation Breeding Project
Sub-Project on Composition or Quality in Rice

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2.1 Introduction

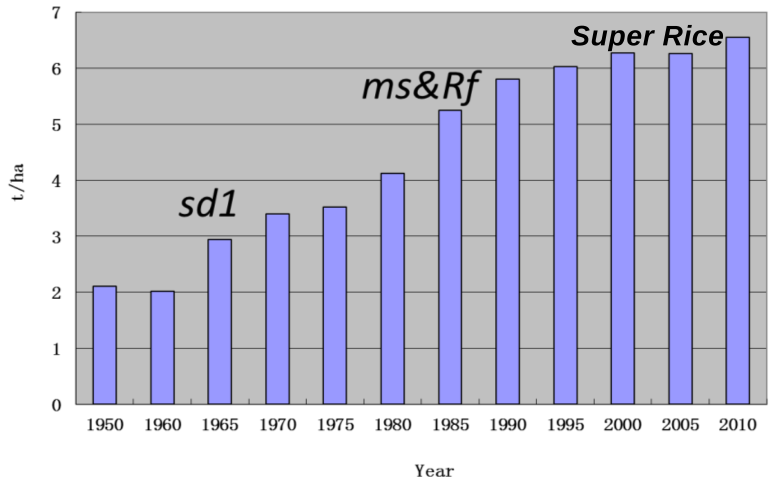
Rice (*Oryza sativa L.*) is one of the most important crops in the world and is a staple food crop for at least 65% of the population of China. The performance of rice sector in terms of production and yield had been very impressive in the most of last six decades. The wide adoption of semi-dwarf varieties expedited the rice yield per unit from 2t/hm^2 in1960s to the level of 3.5t/hm^2 in 1970s. However, the increase of rice production due to the development of semi-dwarf varieties still could not match the rapid increase in population. Exploitation of heterosis in rice became the important choice for getting more rice. Since 1949, the year of founding of the Peoples' republic of China, the rice production has continued to increase along with the rapid growth of the Chinese population. China's total population reached to 1.37 billion people in 2011 and has increased 148.5% compared with the population in 1949. Meanwhile rice yield per unit and total output increased 254.0% and 313.2% as 6.69 t/hm^2 and 200.8 million tons respectively in 2011. However there is only 11.7% increase about the rice sowing area during this period and the rice sowing area is 30.0 million hm^2 in 2011.

Hybrid rice has played an important role in demand of food supply in China. China has made a great effort to increase its rice yield through exploiting the genetic resources of rice. The performance of rice sector in terms of production and yield had been very impressive in the most of last five decades. Hybrid rice that has a yield advantage of 10-20% over the conventional varieties was developed and commercially grown in 1976, which resulted in the increase of yield to over 6.0 t/hm^2. The area under hybrid rice had been increased from 0.14 million hm^2 in 1976 to about 18 million hm^2 in recent years, more than 60% of the total rice planting areas in China (Table 1). In some regions, like Jiangxi and Sichuan province, more than 90% of their rice varieties belong to hybrid rice. Several thousands of hybrid rice varieties were released in the past 50 years. Until now total 96 super rice varieties were confirmed by Ministry of Agriculture (MOA) and were released to the farmers since MOA initiated the super rice breeding program in 1996. The planting area of super rice occupied 25% of total rice planting area and reached 7.3 million hm^2 in 17 provinces in 2011 (Figure 1). These hybrid rice varieties already made important contribution for food security in China.

Table 1. Yearly area of hybrid rice and percentage of the total rice area in China in 1976-2010

Year	Hybrids planting area (m hm^2)	% of total rice area
1976	0.14	0.4
1978	4.34	12.6
1982	5.62	17.0
1986	9.00	27.9
1990	13.62	41.2
1997	17.73	55.8
1999	16.55	52.9
2002	15.48	54.9
2005	19.01	65.5
2008	18.45	63.1
2010	18.05	60.2

Figure 1. Increase of yield in rice through genetic improvement in China



With the great progress of hybrid rice research, the general quality of hybrid rice, especially in terms of head rice rate, chalk-ness and the amylose content etc., has dramatically improved. According to the results in South China Rice Regional Trial in1998- 2004, comparing with the state standard for rice quality, the mean amylose content in every type of hybrids belongs to high quality rice (>3rd grade). In regard to head rice rate (>3rd grade), over 60% single *indica* and late *indica*, over 90% single-season late japonica hybrids reached the standard respectively (Table 2). However, among the 560 hybrid rice combinations developed in 1998-2004, 106 of them reached state standard of the 3rd grade of high quality rice. So the coordination of comprehensive quality is very important to hybrid rice breeding. Hybrid rice breeding technology is different from that for inbred rice breeding. Currently, the most popular male sterility system is the cytoplasmic male sterility (CMS, popularly known in China as the three-line system). This utilizes three different lines, namely a cytoplasmic male sterile line (A line), a maintainer (B line), and a restorer (R line). The quality of B and R line is the key to determine the quality of hybrid rice regarding to three-line system.

Table 2. Performance of hybrids in some quality items in South China Rice Regional Trial in 1998-2004

Type*	Head rice rate (%)		Chalkness (%)		Amylose content (%)		Over 3rd grade	
	Mean	% hybrids reaching the standard	Mean	% hybrids reaching the standard	Mean	% hybrids reaching the standard	No. of hybrids	%
EI (Y)	42.7	21.1	17.3	11.3	19.4	52.1	1	1.4
SI (Y)	57.9	81.0	9.9	30.0	20.7	76.2	44	21.0
LI (Y)	53.9	64.8	9.3	37.3	20.2	85.9	30	21.1
SJ (Y)	70.7	93.1	4.5	58.6	15.5	82.8	17	58.6
EI (S)	48.9	44.4	14.1	8.3	20.6	45.8	0	0.0
LI (S)	62.5	97.2	5.7	55.6	20.8	69.4	14	38.9
Total		66.3		29.6		71.8	106	18.5

*EI(Y or S)─ Early season *indica* rice (Yangtze River or south China)

LI(Y or S)─ Late season *indica* rice (Yangtze River or south China)

SI&J (Y) ─ Single *indica* or *japonica* rice (Yangtze River)

2.2 Objective of the Project

The main objective is to obtain ideal CMS lines and restorers with good quality including adaptive amylose content and to get the optimum irradiation doses of heavy ion beam for early super rice Z7 and late-season inbred rice W2 to create efficient mutant.

2.3 Materials and Methods

Two experiments were carried out in this project during 2008-2010.

2.3.1 Experiment 1 Rice mutation breeding for hybrid rice with high yield and good quality

(1) Mutation breeding of CMS lines

Zhong 3A is a good new indica CMS line with good-grain quality and high disease resistance ability, developed from crossing Zhong 9A to Zhenong996/Jin 23B and successively backcrossing its filial male sterile plants to Zhenong/Jin 23B. It showed stable and complete male sterility, vigorous stigma with high exsertion rate, good disease resistance ability, high combining ability and high outcrossing rate. It had tall and thick stems with good lodging resistance. It was technically identified in Zhejiang Province in September 2005. However, most of the hybrids crossed with Zhong 3A showed high amylose content (>22%). The amylose content of Zhong 3B is 20.1%. Therefore we expect to decrease amylose content of the CMS lines in order to obtain hybrid rice with low amylose content (15-20%) by mutation breeding. In 2008, seeds of Zhong 3B were irradiated with 350Gy and 400Gy from Co-60 gamma irradiator in Hangzhou. Ten M4 lines of Zhong 3 B (maintainer) with low amylose content were selected for further backcross with CMS lines in 2010.

(2) Mutation breeding of restorers

AVF-cyclotron, TIARA, JAEA

Nineteen restorers including CR63, CR84, CR1577 had been collected and irradiated with 250Gy from Co-60 gamma irradiator in Hangzhou in 2006. Six crosses as CR1577(Mutant)/R501, CR1577(Mutant)/R725, Yangdao6/CR1577(Mutant), Shuhui527/CR63(Mutant), CR63(Mutant)/Minghui86, R207/CR84(Mutant) were made and harvested for restorer selection in winter of 2008. Two restorers (ZZ-2 and 07HY-1580) were collected and irradiated with 350Gy and 400Gy from Co-60 gamma irradiator in Hangzhou in 2008. For each treatment 2500 seeds were used. The main objective is to obtain ideal restorers with good quality including adaptive amylose content. Two mutant lines (M_4) from a restorer as CR84 were crossed with CMS lines as Nei 2A, Nei 5A, Nei 6A,, 618A and Zhong 3A respectively in winter of 2009. These hybrids showed good appearance quality, weak potential ability of high yield in summer of 2010. 79 lines of F4 generations were obtained from six combinations between mutant lines with the other restorers through agronomic traits selection. These restorer mutants showed adaptive amylose content. Some of mutants were used as restorer parent for further utilization.

2.3.2 Experiment 2 Seed Irradiation Test by Ion Beam for Rice Mutation Breeding

Through the FNCA cooperation project we initiated heavy ion beam irradiation technology for mutation breeding research in 2010. Two batch of hulled rice seeds were irradiated by the heavy ion beam of the TIARA, JAEA. Two rice cultivars as Z17 and W2 were used. One is a super-high-yielding early-season i*ndica* cultivar; another is a good-quality late-season *indica* cultivar.

The dry seeds of Z17 and W2 were irradiated by carbon ion by the doses of 10, 20, 40, 60, 80, 100, 120Gy. About 150 seeds were packed with plastic Petri dish for each experiment per test-dose. In addition more dry seeds of Z17 were irradiated by carbon ion by the doses of 30, 40, 50Gy respectively, 400 seeds for each irradiation. After irradiation, biological effects were be observed by measurements of the germination rate, survival rate, growth rate and ripening rate. Sowing date, transplanting date and harvesting date was June 10th, July 9th and Oct 12, 2010 respectively. Method of measurement is same to J. Hidema's experiment. After irradiation, the seeds were placed on wet filter paper in petri dish and kept at 30^{o}C for 2 days and then the seeds were planted in plastic-tray containing sterile field soil and placed in the control environmental greenhouse (12hr photoperiod, day/night 27^{o}C/27^{o}C). The germination rate was measured at 2days after planting. The survival rate was measured by counting the viable plants at 2 and 3 weeks after germination. The growth rate (the ratio of the value for plant length of each irradiated plant to the value for plant length of un-irradiated plant) was measured, 30 days after sowing in the control environmental greenhouse. The percentage of grain ripening was measured at 4 months after sowing (maturity stage).

2.4 Results and Discussion

Along with the irradiation dosage increasing, the germination rate, survival rate, growth rate, ripening rate decreased in different extent. Ion beam made some damages to rice seed (Figure 2, 3) . Regarding to Z17, when the irradiation dosage is up to 50Gy, the survival rate and ripening rate make a sharp decrease. When the dosage is down to 20Gy, there is no big difference in all investigated data. Ripening rate significantly decreased at doses of up to about 60Gy, but panicle number significantly increased at doses of up to about 100Gy (Table 3,4, Figure 4, 5). The sterile and low seed setting plants probably kept tillering under water condition in the field. The survival rate of W2 after irradiation decreased sharply (Figure 6).

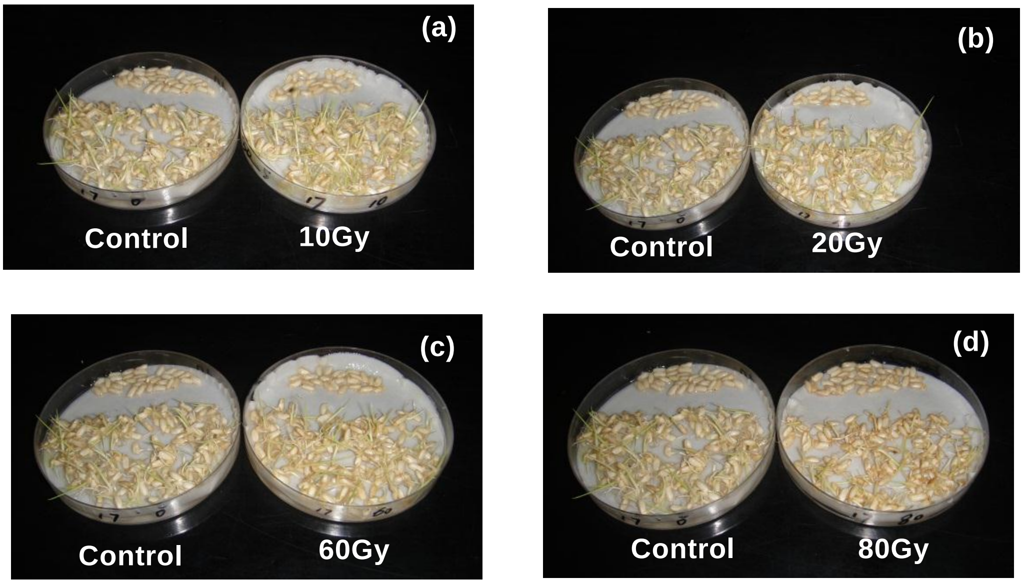


Figure 2. Effect of different dose of ion-beam irradiation of Z17 at day 2.
(a)Germination rate at dose (0Gy and 10Gy), (b)Germination rate at dose (0Gy and 20Gy), (c)Germination rate at dose (0Gy and 60Gy), (d)Germination rate at dose (0Gy and 80Gy).

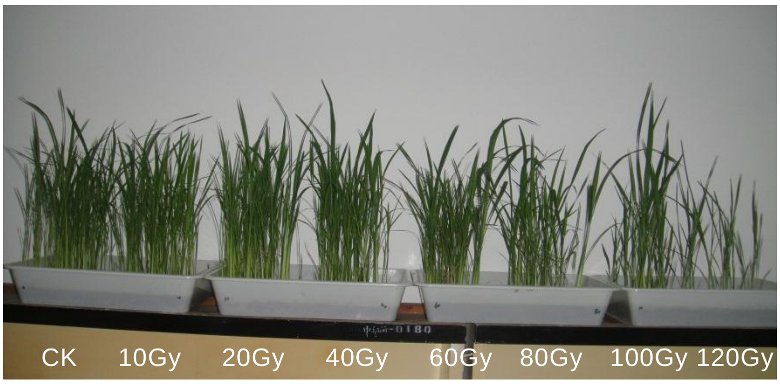


Figure 3. Emergency features of Z17 seedling in a phytotron.

Table 3. Z17 Performance after ion-beam irradiation

Dose (Gy)	Germination rate (%)	Survival rate (%)		Plant Height* (cm)	Growth rate (%)	Percentage of ripening (%)
		2weeks after germination	3weeks after germination			
0 (Control)	87.25	63.09	50.34	30.33	100.00	74.20
10	83.39	63.76	47.65	29.67	97.85	73.30
20	76.16	51.66	35.10	30.19	99.55	61.70
40	73.83	51.01	33.56	30.04	99.05	51.20
60	70.34	46.21	32.41	26.32	86.79	32.40
80	73.51	43.05	26.49	27.09	89.32	13.20
100	72.85	33.11	17.88	28.15	92.82	25.70
120	67.11	20.81	10.74	26.59	87.69	12.80

*: Measured at 30 days after sowing in the control environmental greenhouse.

Table 3 showed the effects of carbon-ion beams on plant height, panicle number, seed number and ripening rate in rice plant. Ripening rate significantly decreased at doses of up to about 60Gy, but panicle number significantly increased at doses of up to about 100Gy. The sterile and low seed setting plants probably kept tillering under water condition in the field.

Table 4. Agricultural traits average performance of Z17 M_1 generation

Dose (Gy)	Plant height (cm)	Panicle number (no./plant)	Seed number (no. /panicle)	Ripening rate (%)
Control	94.1	8.35	150.65	74.2
10Gy	93.6	9.30	150.86	73.3
20 Gy	93.0	8.40	152.24	61.7
40 Gy	93.5	9.85	150.90	51.2
60 Gy	95.0	9.70	170.62	32.4
80 Gy	89.9	9.15	168.08	13.2
100 Gy	90.2	11.25	158.06	25.7
120 Gy	87.9	11.00	164.17	12.8

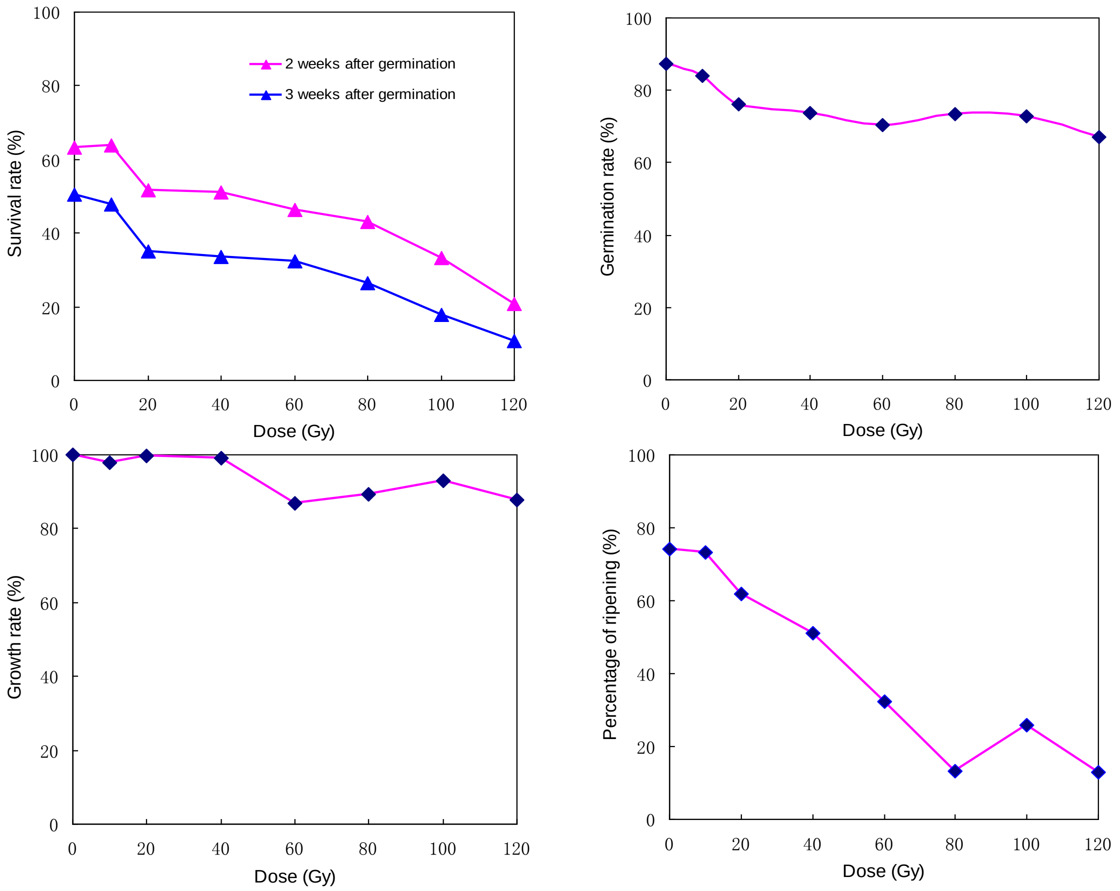


Figure 4. Z17 Dose-response curve for germination rate, survival rate, growth rate and percentage of ripening.

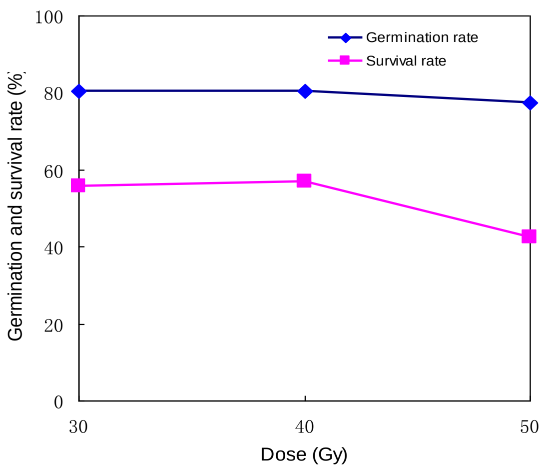


Figure 5. Z17 Dose-response curve (30, 40, 50Gy) for germination rate, survival rate.

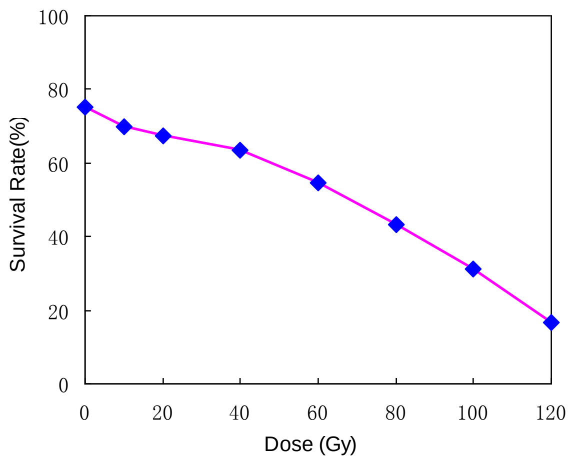


Figure 6. W2 Dose-response curve for survival rate.

2.5 Conclusion

Hulled seeds of Z17, local super rice cultivar, was irradiated with 7 different doses (10, 20, 40, 60, 80, 100, 120Gy) by the heavy ion beam of the TIARA, JAEA in 2010. These materials with 0 doses as control were cultivated in summer of 2010. The germination rate, survival rate, growth rate and percentage of ripening were investigated. These results suggested that the optimum irradiation doses of heavy ion beam for Z7 might be between 30 to 50Gy doses.

2.6 Acknowledgments

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2.7 References

Cao L-Y, Zhan X-D, Chen S-G, Feng Y, Wu W-R, Shen X-H, Cheng S-H, Breeding Methodology and Practice of Super Rice in China. ***Rice Science***, 2010, 17(2): 87-93

Cheng S-H, Cao L-Y, Yang S-H, Zhai H-Q. Forty years' development of hybrid rice: China's experience. ***Rice Science***. 2004, 11, 225-230.

Cheng S-H, Zhuang J-Y, Fan Y-Y, Du J-H, Cao L-Y. Progress in Research and Development on Hybrid Rice: A super-domesticate in China. ***Annals of Botany,*** 2007, 100: 959–966.

Luo J, Tang S-Q, Jiao G-A, Hu P-S. Breeding of Good Quality and Resistance Indica CMS Line Zhong 3A. ***Hybrid Rice***, 2007, 22(1)：13-14

Hidema, J., Yamamoto, M., Kumagai, T., Hase., Sakamoto A., and Tanaka, A. Biological Effects of Carbon Ion on Rice (Oryza sativa L.) ***JAERI-review***.:85-87. (2003)

Yuan L-P. Strategy of hybrid rice breeding. ***Hybrid Rice***, 1987, (1):1-3.