Indian J. Genet., (2022); 82(3): 355-358
doi: 10.31742/ISGPB.82.3.11

ISSN: 0975-6906
www.ijgpb.com

SHORT RESEARCH ARTICLE

Comparative analysis of gamma rays and electron beam in altering rice (*Oryza sativa* L.) grain size

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Abstract

Main objective of this study was to alter the grain size of drought tolerant Anna (R) 4 rice variety, because it fetches less market due to its long slender grain type. Hence to reduce the grain size, the seeds of Anna (R) 4rice were irradiated with physical mutagens namely gamma rays and electron beam. The small grain size mutants from 200, 250, 300 and 350 Gy were advanced to M2 and further generations. The maximum kernel length reduction obtained was 6.04 and 5.71 mm in M2 generation of gamma rays and electron beam respectively. On selection of small grain mutants over generations, the more number of mutants was realisedfrom electron beam in M4 generation especially at 300 Gy, in terms of reduced kernel length, kernel width and grain weight. This offers chance to selecting early maturing and high grain yield per plant over wild type. These findings indicated that mutation approach by electron beam offers high chance of getting small grain size mutants as well as grain yield improvement than gamma rays.

Keywords: Rice, electron beam, gamma rays, grain size, mutation, mutants

Introduction

Rice is one of the most important cereal crops worldwide, and it is consumed by more than half of the world's population. Grain size is a prime breeding target, as it affects both yield and quality (Wang et al. 2015). Since, visual characteristics of rice grains are important search attributes that affect consumers' purchasing decisions and hence grain size used as important selection criteria in varietal improvement program. In rice, grain shape is the key determinant of market value in terms of consumer preference and follows adoption of new varieties by the farmers. Grain length determines appearance and milling, cooking and eating quality of rice (Fan et al. 2009). Consumers of across world prefers long and slender rice or short and fine rice or short and bold rice, which vary region to region.

The hybridization and selection in rice is one of the options to alter grain type. But this process generates variations derived from both parent for all traits and the selection of desirable plant type become complex process. Bao (2014) reported a total of 47 QTLs for grain length. Among which, two genes have been map-based cloned namely GRAIN SIZE 3 (GS3) and GRAIN LENGTH 3 (GL3). Zang et al. (2013) characterised GS2 gene as dominant gene for grain length and grain width. Mutation by irradiation could bring changes in the gene size controlling genes and/or deletion in QTL regions and release the more grain variations.

In India, very few studies are available on use of electron beam as mutagen in rice. The mutagenic effect of radiation is known to be a function of Linear energy transfer (LET), both γ-rays and electron beams have only low LETs of around 0.2 KeV/ μm (Magori et al. 2010). Radiation techniques have generated enormous genetic variations which are a source of phenotypic diversity and are a major driver of evolutionary diversification (Jankowicz-Cieslak et al. 2017; Gowthami et al. 2017; Sao et al. 2020). However, electron beam is administered as a short impulse while gamma

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How to cite this article: Arunachalam P., Lalitha R., Vanniarajan C., Souframanien J. 2022. Comparative analysis of gamma rays and electron beam in altering rice (*Oryza sativa* L.) grain size. Indian J. Genet. Plant Breed., **82**(3): 355-358.

Source of support: Grant No. 35/14/13/2017-BRNS, Govt. of India

Conflict of interest: None.

Received: Dec. 2021 **Revised:** June 2022 **Accepted:** July 2022

Table 1. Mean, range for grain and yield traits in M_2 generation of rice grain mutants

Radiation / Dose	Hundred grain weight (g)		Kernel Length (mm)		Kernel width (mm)		Grain yield per plant (g)	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range
Anna (R) 4	2.58±0.09	2.10-3.00	6.95±0.01	6.60- 6.98	2.04±0.01	2.03-2.05	24.64±0.77	21.00-28.00
Gamma rays								
200Gy	2.42±0.04**	2.10 - 3.10	6.41±0.02**	6.04 - 6.53	2.14±0.03**	1.98 - 2.40	24.07±1.25	11.0 - 39.5
250Gy	2.54±0.04	2.20 -2.90	6.44±0.03**	6.36 - 6.52	2.08±0.02**	1.96 - 2.30	26.9±1.54*	13.8 -42.3
300Gy	2.31±0.06**	2.10 - 3.10	6.39±0.03**	6.20 - 6.51	2.08±0.02**	1.96 - 2.30	26.9±1.34*	13.8- 42.3
350Gy	2.41±0.02**	2.41 -2.50	6.46±0.01**	6.41 - 6.50	2.08±0.02**	2.06-2.16	32.7±1.34**	23.1- 42.4
Electron beam								
200Gy	1.45±0.07**	1.40-1.52	5.79±0.04**	5.78 - 5.80	1.84±0.02**	1.84 - 1.86	12.45±1.69**	10.0 -15.4
250Gy	1.80±0.07**	1.41-2.41	5.94±0.05**	5.72 - 6.37	1.89±0.03**	1.81 - 2.11	14.24±1.88**	6.20 -27.7
300Gy	1.97±0.04**	1.30-3.01	5.92±0.05**	5.71 - 6.53	1.91±0.03**	1.79 -2.26	20.82±1.11**	8.23-45.14
350Gy	2.34±0.09**	2.27-2.47	6.49±0.03**	6.46 - 6.52	1.97±0.02**	1.87 - 2.07	26.74±1.82*	25.40-27.60

Note: *, ** significantly differ from Anna (R) 4 rice at p = 0.05 and 0.01 respectively

Table 2. Mean, range of grain mutants for grain and yield traits in M2, M3 and M4 generations

Generations / Traits	Anna(R)4	Gamma rays mutants		Electron beam mutants	
	Mean	Mean	Range	Mean	Range
M2 generation					
Hundred grain weight (g)	2.58	2.41	2.10 – 3.10	1.94	1.30 – 3.01
Kernel Length (mm)	6.95	6.43	6.04 – 6.53	5.98	5.71 – 6.53
Kernel width (mm)	2.04	2.12	1.96 – 2.40	1.91	1.79 – 2.26
Grain yield per plant (g)	24.64	26.44	11.0 – 42.4	19.93	6.20 – 45.14
M3 generation					
Hundred grain weight (g)	2.46	2.14	1.60 - 2.50	1.61	1.40 - 2.10
Kernel Length (mm)	6.87	6.23	5.50 - 6.46	5.78	5.45 - 6.23
Kernel width (mm)	2.03	2.02	1.86 - 2.12	1.87	1.78 - 2.19
Grain yield per plant (g)	23.68	23.36	13.18 - 31.77	27.11	15.32 - 42.32
M4 generation					
Hundred grain weight (g)	2.57	3.05	2.65 - 3.48	2.45	1.77 - 3.24
Kernel Length (mm)	7.01	6.21	5.90 - 7.00	6.08	5.80 - 6.40
Kernel width (mm)	2.10	2.12	1.95-2.25	2.03	1.95 - 2.70
Grain yield per plant (g)	30.57	33.85	22.48 - 44.36	32.46	16.32 - 52.04

Table 3. Small grain mutants isolatedfrom different generation in rice

Radiation / Dose (Gy)	Gamma rays Mutants (no.)*			Electron Beam Mutants (no.)*		
	M2	M3	M4	M2	M3	M4
200Gy	12	11	7	2	1	1
250Gy	5	3	5	12	4	3
300Gy	6	3	0	63	37	35
350Gy	1	1	0	2	5	5
No. of Mutants	24	18	12	79	47	44
Mean grain yield per plant (g)	25.7	25.9	33.9	20.2	28.8	32.4

* Mutants with < 6.6 mm and > 20 g of grain yield per plant and maturity of < 110 days

irradiation is continuous, due to the fact that electron beam has a higher dose rate compared to γ-rays (Souframanien et al. 2016).

Varieties released for cultivation in drought are generally long bold grain sized type. Though provide maximum grain yield, it reduces the marketability to the marginal farmers.

For an instance, Anna (R) 4 rice variety has ability to tolerate moisture stress and is specially released for rainfed tracts of Tamil Nadu, India. But its long slender grain type is not preferred by the farmers of Tamil Nadu because of low market price. The main aim of this study is to bring grain type alterations in Anna (R) 4 rice cultivar to improve grain qualities by physical mutagens. The ultimate objective is to develop medium slender and high yielding genotypes, to meet market demand that will lead to increased adaptation by farmers with good remunerative price.

The seeds of Anna (R) 4 rice were exposed for gamma rays (^{60}Co) and electron beam (10 MeV) with doses ranged from 100 to 400 Gy with an interval of 50 Gy at Bhabha Atomic Research Centre (BARC), Mumbai. The percent germination of irradiated seeds was assessed *in vitro* by roll towel method, the lethal Dose 50 (LD_{50}) was estimated for gamma rays (376.57 Gy) and electron beam (273.27 Gy) in Anna (R) 4 rice cultivar by Probit analysis (Finney, 1971). Sao et al. (2020) reported that the optimum dose range for gamma ray is 280 to 350Gy, for electron beam is 290 to 330Gy, for X-ray is 200 to 250 Gy and for proton beam is 150 to 200 Gy in short grain aromatic rice.

Based on LD_{50} values, the irradiated seeds (M_0) of 200Gy, 250Gy, 300Gy, 350Gy doses from both the source were raised to generate M_1 generation at Agricultural College and Research Institute, Madurai during October 2017 to January 2018. The seeds of each dose were raised in 20 rows of 4 m length with the spacing of 20 x 15 cm and the recommended agronomical practices were followed to ensure the good crop stand. Two hundred primary panicles were collected from each dose of gamma rays and electron beam irradiated population.

Seeds from 200 primary panicles of each dose were raised in nursery, chlorophyll mutants were observed, healthyseedlings were used to grow M_2 generation during June to October 2018. The total population grown in main field were 12,240 plants in gamma rays and 5,400 plants in electron beam.In M_2 generation, 250 plants were selected based on visual observation of reduced grain size in each dose of gamma rays and electron beam population for further grain analysis.

The precise grain analysiswas carried out in SATAKE Grain scanner - RSQI version 1.26 at Centre of Innovation, Agricultural College and Research Institute, TNAU, Madurai, India. Twenty unbroken rice kernels (after dehulling and before milling) were analysed for each mutant line using the instrument, the average value of kernel length (mm), kernel width (mm), length and breadth ratio was taken for determination of grain shape. Grain shape was determined as per classification of Standard Evaluation System (IRRI 2013). Further hundred grain weight (g) of selected grain size mutants was recorded.

Stringent selection was exercised on grain mutants, 110 mutants (31 from gamma rays and 79 from electron beam) were advanced from M_2 to M_3 generation with early maturity (< 110 days), single plant yield (>20 g) and reduced grain size in terms of kernel length, kernel breadth and grain weight. The M_3 generation was raisedduring February to May 2019. Based on the performance evaluation and grain characters, 18 gamma rays and 47 electron beam mutantswere advanced to M_4 generation and was raised during *kharif* 2019.

Wide spectrums of morphological mutants were observed in both gamma rays and electron beam irradiated M_2 population for growth habit, flowering and grain characters. Overall frequency of morphological mutant was high in electron beam (0.05) than gamma rays (0.03). In M_2 population, 110 reduced grain size mutants (31 from gamma rays and 79 from electron beam) were sorted out and most of them are medium slender grain type. In gamma rays, the kernel length reduction was up to 6.04 mm (12.82%), whereas in electron beam it was5.71mm (17.74%) from Anna (R) 4 rice (6.95 mm). In electron beam mutants,the mean kernel width was significantly reduced and to the maximum of 1.79 mm as compared to wild type of 2.04 mm (Table 1). In contrast, the mean kernel width was significantly increased in gamma rays compared to wild type. These results indicated that, the electron beam irradiation found to be efficient in reducing grain size in terms of reduced kernel length and kernel width.This was evident from less hundred grain weight in electron beam (1.3 to 3.01 g) than gamma rays (2.1 to 3.1 g) in M_2 mutants. The more number of small grain size mutants were obtained from 200 to 300 Gy of electron beam.

Further in M_2 generation, more variation for the grain yield per plant was realised among small grain size mutants. In gamma rays mutants, the grain yield per plant recorded 11.0 to 42.4 g; whereas, in electron beam mutants it ranged from 6.20 to 45.14 g. These results suggested there is a higher chance of electron beam mutants could produce desirable grain types to improve the grain quality in rice along with yield improvement.

The high frequency of medium slender grain type mutants with the kernel length of less than 6.6 mm was recovered from electron beam than gamma rays induced mutation in M_2 generation. Among the doses 300 Gy produced high number of smallgrain mutants from electron beam source (Table 3).The grain-type mutant induction was higher in the electron beam treated population than in gamma rays treated population in rice (Gowthami et al. 2021).The reduction in grain size is might be due to the loss of function of SMALL GRAIN 1 (SMG1)/ (OsMKK4) and OsMAPK6 in MAPK cascades leading to small grains by these gene restricts cell proliferation in spikelet (Duan et al. 2014; Liu et al. 2015). The loss of function of XIAO also results in typical BR-related phenotypes and reduced grain length (Jiang et al. 2012). The spikelet hull development is coordinately regulated by cell proliferation and cell expansion, the reduced grain size effected by controlling the genes involved in the cell expansion (Liet al. 2018).

The selection for small grain size, early duration and grain yield from M_2 generation resulted reduction in mean kernel length of 6.23 mm (M_3) and 6.21 mm (M_4) in gamma rays and 5.78 mm (M_3) and 6.08 mm (M_4) in electron beam (Table 2). The smaller grain size realised is 1.77 g as hundred grain weight in electron beam and 2.65 g in gamma rays in M_4 generation. The single plant yield ranged from 13.18 to 31.77 g (M_3) and 22.48 to 44.36 g (M_4) in gamma rays, 15.32 to 42.32 g(M_3) and 16.32 to 52.04 g (M_4) in electron beam mutants.

The mean performance of grain yield per plant was comparable from both the sources of radiation across generations (Table 3). But more number of small grain mutants with high grain yield per plant and short duration (<110 days) was obtained from electron beam (44 no.) than gamma rays (12 no.) in M_4 generation.The high narrow-sense heritability was reported for grain length, grain width and grain weight over generations (Kato 1990 and Moeljopawiro 2002). Hence early generation selection response was obtained for grain characters to bring the changes in desirable direction.

The irradiation approach helps to impart desired grain type to rice cultivar as well as grain yield improvement, especially more chance from electron beam. This is due to a wide range of genetic variations realised in M_2, followed by precise phenotyping of grain traits, intense selection pressure excised and inherent property of high heritability associated with them. These results throw the scope to develop a rice variety with desired consumer preference that fetches better market price to the farmer.

Authors' contribution

Conceptualization of research (PA, CV, JS); Designing of the experiments (PA, CV, JS); Contribution of experimental materials (PA, JS); Execution of field/lab experiments and data collection (PA, RL); Analysis of data and interpretation (PA, RL); Preparation of the manuscript (PA).

Acknowledgements

Authors extend their sincere thanks to Government of India, Board of Research in Nuclear Sciences (BRNS), Department of Atomic Energy, Mumbai, for providing research grant [No.35/14/13/2017-BRNS] for this study.

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