Effectiveness and Efficiency of Gamma Rays and Electron Beam in $M_2$ Generation of Anna (R) 4 Rice Mutants

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ABSTRACT

A study was conducted to assess the chlorophyll and viable mutation frequency in $M_2$ generation of Anna (R) 4 rice variety induced through gamma rays and electron beam radiations with different dosages viz., 200Gy, 250Gy, 300Gy and 350Gy. The results showed that chlorophyll and viable mutation frequency was higher in electron beam (8.62 and 22.30) compared to gamma rays (8.11 and 13.40). The electron beam was found to have higher mean effectiveness (0.059) compared to gamma rays (0.04), while higher efficiency (0.723) was observed in gamma rays and fairly less efficiency was reported in electron beam (0.540). However, higher frequencies of viable and useful mutants were obtained in electron beam. Therefore the electron beam can be used as an alternate source for physical mutagen to produce useful mutants in rice improvement.

Key words: Chlorophyll mutants, Effectiveness, Efficiency, Electron beam, Gamma rays, Rice.

INTRODUCTION

Rice is one of the pivotal crop in agriculture, feeding more than half of the world's population. It is the most diversified crop due to its adaptation to wide range of geographical and climatic regimes. The creation of variability in quantitatively and qualitatively inherited traits in various crops through induced mutation has been extensively studied by Maluszynski et al. (1995) and Muduli and Mishra (2007). Chlorophyll mutations are one of the reliable indices for evaluating the mutagenic induced genetic alterations of the mutagen treatments used on the plant ideotype (Chaturvedi and Singh, 1990). Nuclear gene mutations or extra chromosomal mutations might result in chlorophyll deficient mutations (Levine 1972, Walles 1973 and Wildman 1973). In general any mutational event may bring large or small changes in the phenotype of the plant. The changes in macro mutants have highest significance in plant breeding because they may sometimes give a desired phenotype. Electron beam radiation has little influence on the function of plasma membrane and protein, while it results in gene mutation through inducing much DNA damage of single strand breaks (SSB) and double strand breaks (DSB). The G-value for DSB formation of electron beam radiation in aqueous solution was 5.7 times higher than that caused by $^60$Co-gamma rays (Zhu et al. 2008). In the present study attempt has been made to investigate the effectiveness and efficiency of induced mutation by gamma rays and electron beam in Anna (R) 4 rice variety with respect to chlorophyll and viable mutation frequency.

MATERIALS AND METHODS

The rice variety Anna(R) 4 seeds were treated with gamma rays and electron beam with four different doses (200Gy, 250Gy, 300Gy and 350Gy) at Bhabha Atomic Research Centre, Mumbai for gamma rays (Zhu et al. 2008). In the present study attempt was made to investigate the effectiveness and efficiency of induced mutation by gamma rays and electron beam in Anna (R) 4 rice variety induced through gamma rays and electron beam radiations with different dosages viz., 200Gy, 250Gy, 300Gy and 350Gy. The electron beam was found to have higher mean effectiveness (0.059) compared to gamma rays (0.04), while higher efficiency (0.723) was observed in gamma rays and fairly less efficiency was reported in electron beam (0.540). However, higher frequencies of viable and useful mutants were obtained in electron beam. Therefore the electron beam can be used as an alternate source for physical mutagen to produce useful mutants in rice improvement.

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generation, various chlorophyll mutants such as albino, xantha, chlorina, striata, tigrina and albomaculata were observed (Gustafsson, 1940; Blixt and Gottasckal, 1975) periodically at the stage of eight to fifteen days after sowing. M₃ population and mutated M₄ panicle progenies form the M₅ seedlings. The progeny of chlorophyll mutations (mutants) were worked out as per the formulae suggested by Madhusudana Rao and Mannama Rao (1979). M₃ panicle progeny basis = 

Total number of mutations / No. of M₃ panicle progeny

M₄ population = 

No. of mutants / No. of M₃ plants

Mutants per mutation = 

No. of mutants from mutated panicle progeny / No. of plants from mutated panicle progeny x 100

In M₄ generation, viable mutations were observed throughout the life span viz., dwarf, tall, leaf shape and size, early flowering, late flowering, number of tiller formation, floral morphology, spikelet formation, grain colour and grain shape were observed in both gamma rays and electron beam. Based on the formulae suggested by Konzak et al. (1965), data obtained in M₅ generation (Injury and Lethality) and mutation frequencies in M₅ generation (Chlorophyll and viable mutants) of Anna (R) 4 rice were used to determine the effectiveness and efficiency of gamma rays and electron beam.

Mutagenic effectiveness = 

Mutation frequency / Dose (Gy)

Mutagenic efficiency = 

Mutation frequency / Biological damage

Mutation rate

Mutation rate provides the knowledge of mutations induced by a particular mutagen irrespective of dose or concentration.

Mutation rate = Sum of values of effectiveness or efficiency of particular mutagen / Number of treatments of a particular Mutagen

RESULTS AND DISCUSSION

Chlorophyll mutants

The genetic effects due to mutation were easily assessed by chlorophyll mutations in earlier stages of plants. They occurred due to weakening of chlorophyll biosynthesis activity, degrading the chlorophyll content and bleaching due to deficiency of carotenoids (Bevins et al. 1992) and also due to chlorophyll development genes (Reddy and Annadurai, 1991).

In M₃ panicle progeny basis, the per cent of chlorophyll mutants were increased with increase in dose with an exception of 350Gy in electron beam (Table 1). The electron beam treatment recorded higher per cent of chlorophyll mutants when compared to gamma rays. The maximum chlorophyll mutants were observed on panicle progeny at 300Gy (101.50) of electron beam compared to gamma rays. The M₃ plant population also showed similar trends as in M₄ panicle progeny with an exception of 250Gy. The treatment with electron beam recorded highest per cent of mutation at 350Gy (2.92) than all other treatment followed by gamma rays at 250 Gy (2.74). The mutants per mutation showed maximum of 30.20 per cent in 300 Gy of gamma rays treatment followed by 250Gy (25.28). In electron beam, 300Gy recorded maximum of 17.59 followed by 250Gy (14.70).

Based on the studies on M₃ panicle to progeny row showed higher per cent of chlorophyll mutants but it will give inflated figures compared to M₄ population. The M₄ population is more reliable as it gives an actual per cent of mutants in M₅ generation. From the breeder point of view also, the frequency of mutants expressed in percentage of mutants on M₄ population basis is more realistic and helpful (Gaul, 1964). Therefore, in this study spectrum of chlorophyll mutants were worked out based on M₄ population to obtain valid results about chlorophyll mutants.

Spectrum of chlorophyll and morphological mutants

In this study, wide range of chlorophyll mutants recorded from 0.78% to 2.5% and 1.13% to 2.92% for gamma rays and electron beam respectively. Dose dependent increase in frequency of chlorophyll mutants was noticed in gamma rays (except 250Gy) and electron beam. The high incidence of chlorophyll mutations induced by electron beam, in the present study, may be due to its specificity to affect certain regions of chromosomes. Chlorophyll development seems to be controlled by many genes located on several chromosomes which could be adjacent to centromere and proximal segment of chromosome.

Among the different types of chlorophyll mutants, albino was occurred more frequently at higher doses at 350 Gy (80.62% and 77.04%) followed by chlorina (17.05% and 13.78%) for both gamma rays and electron beam. Other chlorophyll mutants like xantha, striata, tigrina and albomaculata were observed in lower doses. The higher incidence of albino mutants than xantha and viridis type induced by radiation were also reported by Mikaelsen et al. (1968) ando (1970) and Awan and Bari (1979). This agrees well with the results obtained in the study (Table 2). The occurrence of mutation in one or other doses is apparently due to polygene for chlorophyll formation and also sensitivity of genes governing chlorophyll (Gaul, 1964).

Wide spectrums of morphological mutants were observed in both gamma rays and electron beam of M₅ generation. The frequency of morphological mutants increased with increase in dose except at the dosage of 350Gy which showed drastic reduction in both the treatment. Overall morphological mutant was high in electron beam (22.30) than gamma rays (13.40). The induced morphological mutants in different crop plants were studied by different scientists concluded that the occurrence of
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### Table 1: Percent of chlorophyll mutations on M$_1$ panicle progenies and M$_2$ plant populations basis of gamma rays and electron beam treated Anna(R) 4 rice cultivar.

| Doses (Gy) | Gamma rays induced mutants (%) | Electron beam induced mutants (%) |
|------------|--------------------------------|----------------------------------|
|            | M$_1$ plants per panicle mutation | M$_2$ plant population mutation | |
| 200Gy      | 28.0                           | 0.80 (7186)                     | 9.81 [571]          |
| 250Gy      | 68.5                           | 2.74(5002)                      | 25.28 [542]         |
| 300Gy      | 59.5                           | 2.34(5080)                      | 30.20 [394]         |
| 350Gy      | 64.5                           | 2.25(5742)                      | 25.20 [512]         |
| Treatment mean | 55.13                          | 2.03                           | 22.62               |

Note: *Scoring of 200 M$_1$ panicle progenies.
(-) values in parentheses is number of M$_2$ plants observed.
[-] values in parentheses indicates number of plants in mutated plant progeny.

### Table 2: Spectrum of chlorophyll mutants obtained from Anna(R) rice mutants in M$_2$ generation.

| Doses (Gy) | Gamma rays | Chlorophyll mutants (Relative percentage)* |
|------------|------------|-------------------------------------------|
|            |            | M$_2$ plant observed | No. of chlorophyll mutants | Albino | Xantha | Chlorina | Striata | Tigrina | Albmacculata |
| 200Gy      | 7186       | 56                         | 19 (33.90)                  | 10(17.85) | 56 (54.74) | 6(4.38) | 75(54.42) | 137 | 441 |
| 250Gy      | 5002       | 137                        | 75 (54.74)                  | 6(4.38)  | 56 (54.42) | 6(4.38) | 75(54.42) | 137 | 441 |
| 300Gy      | 5080       | 119                        | 107(89.92)                  | 4(3.36)  | 8(6.72)     | 0       | 0       | 0
| 350Gy      | 5742       | 129                        | 104(80.62)                  | 3(2.33)  | 22(17.05)   | 0       | 0       | 0
| Treatment mean | 23010 | 441                        | 305                         | 23       | 105           | 4       | 1       | 3

### Table 2: Spectrum of chlorophyll mutants obtained from Anna(R) rice mutants in M$_2$ generation.

| Doses (Gy) | Gamma rays | Chlorophyll mutants (Relative percentage)* |
|------------|------------|-------------------------------------------|
|            | Electron beam | M$_2$ plant observed | No. of chlorophyll mutants | Albino | Xantha | Chlorina | Striata | Tigrina | Albmacculata |
| 200Gy      | 7890       | 89                         | 59(66.29)                   | 6(6.74)  | 16(17.97)   | 0       | 0       | 8(8.98)
| 250Gy      | 7524       | 163                        | 105(64.42)                  | 13(7.98) | 10(6.13)    | 11(6.75) | 17(10.43) | 7(2.49)
| 300Gy      | 8435       | 203                        | 70(34.48)                   | 0       | 82(40.39)   | 10(4.93) | 39(19.21) | 2(0.99)
| 350Gy      | 6710       | 196                        | 151(77.04)                  | 2(1.02)  | 27(13.78)   | 12(6.12) | 4(2.04)   | 0
| Treatment mean | 30559 | 651                        | 385                         | 21       | 135           | 33      | 60       | 17

Note: *Values in parentheses indicates relative percent; **Values in parentheses indicates number of mutants observed

Altered phenotypes was due to the chromosomal breakage, disturbed auxin synthesis, disruption of mineral metabolism and accumulation of free amino acids (Sharma and Sharma 1979, Goyal and Khan 2010, Gunkel and Sparrow 1961, Blixt 1972, Toker and Cagirgan, 2004).

Effectiveness and efficiency

In M$_2$ generation, based on chlorophyll mutants the effectiveness was calculated for both the treatments of gamma rays and electron beam (Table 3). The results showed that the effectiveness based on chlorophyll mutation frequency was recorded high at 250 Gy for gamma rays (0.011) and electron beam (0.009). Based on viable mutation frequency 300 Gy of electron beam was the most effective (0.033) as compared to gamma rays. The overall mutation frequency was recorded higher in electron beam (0.059) as compared to gamma rays (0.049). The reduction of effectiveness while increase with higher dosages in both the treatments indicated that the mutations are independent events and their rate of occurrences is not proportional to
Effectiveness and Efficiency of Gamma Rays and Electron Beam in M<sub>2</sub> Generation of Anna (R) 4 Rice Mutants

Table 3: Effectiveness of gamma rays and electron beam in Anna(R) 4 rice.

| Treatment       | M<sub>2</sub> plants observed (Nursery) | No. of chlorophyll mutants | M<sub>2</sub> plants observed (Main field) | No. of viable mutants | Chlorophyll mutation frequency (%) | Viable mutation frequency (%) | Effectiveness | Mc/Gy | Mv/Gy | Average |
|-----------------|----------------------------------------|----------------------------|-------------------------------------------|-----------------------|-----------------------------------|------------------------------|----------------|--------|-------|---------|
| Gamma rays (GR) |                                        |                            |                                           |                       |                                   |                              | 200            | 0.04   | 0.016 | 0.010  |
| 200             | 7186                                   | 56                         | 3633                                      | 96                    | 0.78                              | 3.14                         | 0.004         |        |       |         |
| 250             | 5002                                   | 137                        | 3355                                      | 126                   | 2.74                              | 4.12                         | 0.011         |        |       | 0.014   |
| 300             | 5080                                   | 119                        | 2720                                      | 138                   | 2.34                              | 4.51                         | 0.008         |        |       | 0.011   |
| 350             | 5742                                   | 129                        | 2532                                      | 50                    | 2.25                              | 1.63                         | 0.006         |        |       | 0.006   |
| Overall (GR)    | 23010                                  | 441                        | 12240                                     | 410                   | 8.11                              | 13.40                        | 0.03          | 0.05   | 0.04   |
| Mutation rate   | -                                      | -                          | -                                         | -                     | 2.03                              | 3.35                         | 0.007         |        |       | 0.010   |
| Electron beam (EB) |                                      |                            |                                           |                       |                                   |                              | 200            | 0.06   | 0.027 | 0.016  |
| 200             | 7890                                   | 89                         | 1506                                      | 73                    | 1.13                              | 5.41                         | 0.006         |        |       | 0.015   |
| 250             | 7524                                   | 163                        | 1460                                      | 75                    | 2.17                              | 5.56                         | 0.009         |        |       | 0.015   |
| 300             | 8435                                   | 203                        | 1278                                      | 134                   | 2.41                              | 9.93                         | 0.008         |        |       | 0.021   |
| 350             | 6710                                   | 196                        | 1156                                      | 19                    | 2.92                              | 1.41                         | 0.008         |        |       | 0.006   |
| Overall (EB)    | 30559                                  | 651                        | 5400                                      | 301                   | 8.62                              | 22.30                        | 0.031         | 0.086  | 0.059  |
| Mutation rate   | -                                      | -                          | -                                         | -                     | 2.16                              | 5.60                         | 0.008         |        |       | 0.015   |

Table 4: Efficiency of gamma rays and electron beam in Anna(R) 4 rice.

| Treatment       | Chlorophyll mutation frequency (%) | Viable mutation frequency (%) | Plant survival reduction (%) in M<sub>1</sub> (L) | Seedling height reduction (%) in M<sub>1</sub> (I) | Efficiency Chlorophyll mutants | Efficiency Viable mutants |
|-----------------|------------------------------------|------------------------------|-----------------------------------------------------|--------------------------------------------------|------------------------------|--------------------------|
| Gamma rays      |                                    |                              |                                                     |                                                   | Mc/L                        | Mc/I                    | Average         | Mv/L | Mv/I | Average |
| 200             | 0.78                               | 3.14                         | 12.24                                               | 11.40                                             | 0.064                       | 0.068                   | 0.066           | 0.256 | 0.275 | 0.266   |
| 250             | 2.74                               | 4.12                         | 20.41                                               | 26.95                                             | 0.134                       | 0.102                   | 0.118           | 0.202 | 0.153 | 0.177   |
| 300             | 2.34                               | 4.51                         | 14.29                                               | 28.14                                             | 0.164                       | 0.083                   | 0.123           | 0.316 | 0.160 | 0.238   |
| 350             | 2.25                               | 1.63                         | 51.02                                               | 31.24                                             | 0.044                       | 0.072                   | 0.058           | 0.032 | 0.052 | 0.042   |
| Overall (GR)    | 8.11                               | 13.40                        | 97.96                                               | 97.73                                             | 0.406                       | 0.325                   | 0.366           | 0.806 | 0.641 | 0.723   |
| Mutation rate   | 2.03                               | 3.35                         |                                                      |                                                   | 0.101                       | 0.081                   | 0.091           | 0.201 | 0.160 | 0.181   |
| Electron beam   |                                    |                              |                                                     |                                                   | 0.116                       | 0.068                   | 0.066           | 0.256 | 0.275 | 0.266   |
| 200             | 1.13                               | 5.41                         | 38.78                                               | 32.93                                             | 0.029                       | 0.034                   | 0.032           | 0.139 | 0.164 | 0.152   |
| 250             | 2.17                               | 5.56                         | 42.86                                               | 34.13                                             | 0.051                       | 0.064                   | 0.057           | 0.130 | 0.163 | 0.146   |
| 300             | 2.41                               | 9.93                         | 48.98                                               | 44.31                                             | 0.049                       | 0.054                   | 0.052           | 0.203 | 0.224 | 0.213   |
| 350             | 2.92                               | 1.41                         | 53.06                                               | 44.91                                             | 0.055                       | 0.065                   | 0.060           | 0.027 | 0.031 | 0.029   |
| Overall (EB)    | 8.63                               | 22.30                        | 183.67                                              | 156.28                                            | 0.184                       | 0.217                   | 0.201           | 0.498 | 0.582 | 0.540   |
| Mutation rate   | 2.16                               | 5.57                         |                                                      |                                                   | 0.046                       | 0.054                   | 0.050           | 0.125 | 0.146 | 0.135   |

The increasing strength of mutagen concentrations. Mutation rate was calculated for gamma rays and electron beam based on effectiveness of Anna(R) 4 mutants showed that electron beam treatment have higher rate of mutation (0.015) than gamma rays. While based on efficiency the mutation rate was maximum in gamma rays (0.181) than electron beam (0.135).

The efficiency estimated for chlorophyll and viable mutants based on biological damage showed that gamma rays have higher efficiency compared to electron beam in Anna(R) 4 mutants (Table 4). Based on lethality, gamma rays recorded maximum efficiency at 300Gy (0.164) and for seedling injury at 250Gy (0.102). The biological damage was lesser in lower and moderate dosages results in higher efficiencies which increased with increase in the strength of the mutagen treatments. Similar results of higher effectiveness and efficiency at the lower and moderate concentrations of mutagens were already reported by Khan and Tyagi (2010) in *Glycine max* and Wani *et al.* (2011) in *Vigna radiata*. Although, higher efficiency was recorded in gamma rays, the maximum number of useful mutants was obtained in electron beam. Spectrums of morphological mutants were observed common in both gamma rays and electron beam. Early flowering, high tillering habit and grain type mutant were recorded high in electron beam (0.009, 0.002, 0.015) than gamma rays (0.005, 0.001, 0.002). In grain type mutant, grain length ranged from 5.70 to 6.53mm for electron beam mutants and in gamma rays 6.04 to 6.53mm were obtained in the study as compared to Anna (R) 4 rice (6.92 mm).
CONCLUSION
The frequency of chlorophyll mutants worked out on the basis of Mₙ population is more realistic to compare mutagen effect than other methods. The electron beam (0.021) produce more number of chlorophyll mutants compared to gamma rays treatment (0.019) and among the chlorophyll mutants, albino mutants were recorded higher level in both the treatment. The effectiveness and efficiency of radiation based on chlorophyll and viable mutants, the electron beam showed more effectiveness than efficiency in mutation rate. But electron beam have higher tendency to produce useful mutants like early flowering, tillering habit and grain type mutants from Anna(R) 4 rice cultivar.

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REFERENCES
Ando, A. (1970). Mutation induction in rice by radiation combined with chemical protectants and mutagens. In: Rice Breeding with Induced Mutations II. IAEA. Vienna. P. 1-5.
Awan, M.A and Bari, G. (1979). Mutagenic effects of fast neutrons and gamma rays in rice. The Nucleus. 16: 33-38.
Bevins, M., Yang, C. M., Markwell, J. (1992). Characterization of chlorophyll deficient mutant of sweet clover (Melilotus albus). Plant Physiology and Biochemistry. 30: 327–331.
Blixt, S. (1972). Mutation genetics in Pisum. Agric. Hort. Genet. 30: 1-293.
Blixt, S. and Gottascalk, W. (1975). Mutation in leguminosae. Agric. Hort. Genet. 33: 33-85.
Chaturvedi and Singh, V.P. (1990). Mutation breeding in pigeonpea. In: Genetic Improvement of Pulse Crops. Vol. I. [Jafar Nizam; Irfan A. Khan and S.A. Farooq (eds)], Premier Publishing House, 800 Kothi Hyderabad.
Gaul, H. (1964). Mutations in plant breeding. Radiation Botany. 4: 155-232.
Goyal, S. and Khan, S. (2010). Differential response of single and combined treatment in moist seeds of urdbean. Indian Journal of Botanical Research. 6(1-2): 183-188.
Gurkhal, J.E. and Sparrow, A.H. (1961). Ionizing radiations: biochemical, physiological and morphological aspects of their effect on plants, in: Encyclopedia of Plant Physiology, [W. Ruhland (ed.),] Springer, Berlin, p. 555-611.
Gustafsson, A. (1940). The mutation system of the chlorophyll apparatus. (K. Fysiogr. Sällsk. Lund Förh.) N.F. 51, 1–40.
Khan, M.H. and Tyagi, S.D. (2010). Induced morphological mutants in soybean [Glycine max (L.) Merrill]. Frontiers of Agriculture in China. 4(2): 175-180.
Konzak C.F., Nilan, R.A., Wagner, J., Foster, R.J. (1965). Efficient chemical mutagenesis. Radiation Botany 5 (Suppl.): 49-70.
Levine, R.P. (1972). Interactions between nuclear and organelle genetic systems. In: Evolution of Genetic systems. Brook Haven Symposium Biology. 23: 503-533.
Madhusudana Rao, G. and Mammad Rao, V. (1983). Mutagenic efficiency, effectiveness and factor of effectiveness of physical and chemical mutagens in rice. Cytologia. 48: 427-436.
Maluszynski, M. Ahlloowalia, B.S. Sigurbjornsson, B. (1995). Application of in vivo and in vitro mutation techniques for crop improvement. Euphytica. 85(1-3): 303–315.
Mikaelens, K., Kiss, I., Osone, K. (1968). Some effects of fast neutrons and gamma radiations on rice. In: Neutron Irradiation of Seeds II, IAEA, Vienna. p. 49-54.
Muduli, K.C. and Mishra, R.C. (2004). Efficacy of mutagenic treatments in producing useful mutants in finger millet (Eleusine coracana) Gaertn. Indian Journal of Genetics and Plant Breeding. 67: (3): 232- 237.
Reddy, V.R.K. and Annadurai, M. (1991). Chlorophyll mutants in lentil. Frequency and segregation. Cytology and Genetics. 26: 31-37.
Sharma, S.K. and Sharma, B. (1979). Leaf mutations induced with NMU and gamma rays in lentil (Lens culinaris Medik.). Current Science. 48: 916-917.
Toker, C. and Cagirgan, M.I. (2004). Spectrum and frequency of induced mutations in chickpea. Int. Chickpea and Pigeonpea Newsletter. 11: 8-10.
Walles, B. (1973). Plastid structures and mutations. In: Structure and Function of Chloroplasts, Gibba Med, Springer Verlag New York, p. 51-55.
Wani, M.R., Khan, S., Kozgar, M.I. (2011). Induced chlorophyll mutations. I. Mutagenic effectiveness and efficiency of EMS, HZ and SA in mungbean. Frontiers of Agriculture in China. 5(4): 514-518.
Wildman, S.G. (1973). An approach towards ascertaining the function of chloroplast DNA in tobacco plants. In: Autonomy of Bio-genesis, [Linnae N K and Smillie A W (eds)], AE Rube Co. New York.
Zhu H, Xu J, Li S, Sun X, Yao S., Wang S. (2008). Effect of high energy- pulse-electron beam radiation on biomolecules. Science in China Series B-Chemistry. 51: 86-91.