Gamma-ray radiation and sodium azide (NaN_3) mutagenic efficiency in rice

Akihiko Ando[1] and Ricardo Montalván*[2]
[1]Departmento de Genética, Escola Superior de Agricultura "Luiz de Queiroz", Universidade de São Paulo, Caixa Postal 83, CEP 13400-970, Piracicaba - SP, Brasil**;** [2]Embrapa Soja, Caixa Postal 231. CEP 86001-970, Londrina-Pr, Brasil. (* Corresponding Author. E-mail: ricardois@operamail.com)

ABSTRACT

Rice *(Oriza sativa L.)* cultivar IAC-1246 seeds were treated with 10, 15, 20 and 30 Krads of gamma-rays and sodium azide (SA) concentrations of 0.5, 1.0 and 5.0 mM to study their efficiency in inducing chlorophyll mutations. Combined treatments incremented the damages when compared to single treatments, especially for seedling height and M_1 panicles fertility. Treatments with SA showed a higher frequency of chlorophyll mutations than the gamma-ray treatments, both in single and combined treatments. The gamma-ray spectrum was different for the various types of mutations, whether in individual or combined treatments. The SA efficiency was higher than that of gamma - rays at 1.0 and 5.0 mM concentrations and lower at 0.5 mM. On average, the additive effect of the mutagenic combinations was more evident than the frequencies of the mutations.

KEY WORDS: Induced mutation, breeding.

INTRODUCTION

The presence of genetic variability is necessary for crop improvement. The variability available to the breeder comes from spontaneous or artificially induced mutations.

Plant breeding involves procedures which increase genetic variation, select desirable genotypes, evaluate selected genotypes, and finally, multiply and release new cultivars.

In mutation breeding, the enhancement of the genetic variation is made through the influence of mutagens. Despite the advantages and limitations of this method, it has been applied in the development of numerous improved cultivars, in different crops, such as wheat, rice, barley, soybean, lupines, vegetables, ornamentals, etc. Several traits have been subjected to mutation breeding: yield, lodging resistance, disease resistance, maturity, culm length, etc. These facts were evaluated by Fehr (1987) who reported that artificial mutation can be a practical mean to achieve genetic improvement in crop species.

Artificial induction of mutations is done through the use of physical and/or chemical mutagens which enlarge the mutation frequency, when compared to the spontaneous occurence. However, for extensive use of these mutants in plant breeding, high production efficiency is essential. This means that the utility of any mutagen depends not only on its effectiveness (mutation factor / dose) but also on its efficiency. The effectiveness of a mutagen has no practical implications since radiations and chemical mutagens are relatively inexpensive. On the other hand, lower levels of mutagens efficiency can limit their uses. Mutagenic efficiency is the production of desirable changes which are free from associations with undesirable genetic alterations. This is generally measured by the proportion of the mutation frequency in relation to damages associated to mutagenic treatments such as: height reduction, chromosomes breakages, sterility, lethality, etc. (Konzak et al., 1965; Gaul et al., 1972).

The possibility of inducing new variability by mutagenic agents is, therefore, of great interest to

genetic improvement. This interest becomes even greater when associated with the possibility of assessing qualitatively and visibly the increases in mutation frequency through chlorophyll mutations.

Mutagenic treatments with two different combined mutagens have ben proposed by many researchers as Ando (1970), to increase the effectiveness and efficiency of mutation induction. The question whether or not combined mutagens have additive effects on the mutation frequency, and not on the physiological damages that they originate, remains unwered. Recently, some combinations of mutagens have been tested in rice: gamma-rays combined with EMS (Rao, 1977), with HA, NMU and MMU (Rao and Rao, 1983); with SA (Reddi and Rao, 1988) and with EMS and DES (Kaul and Bhan, 1977).

This study aimed at assesing the effect of gamma-rays and SA in both single and combined treatments on the mutagenic efficiency and spectra of chlorophyll mutations in rice.

MATERIAL AND METHODS

Five hundred dried and dormant seeds of Brazilian rice, variety IAC-1246, (13% $\pm$ 1,0 moisture) were irradiated with 10, 15, 20 and 30 Kr gamma-rays at 139,3 Kr/hour with a Co^{60} source, at the Centro de Energia Nuclear na Agricultura, in Piracicaba, São Paulo.

For the sodium azide treatment, seeds were treated with 250 ml of freshly prepared 0.5, 1.0 and 5.0 mM of SA (buffered at pH 3) solution for 8 hours with continuous shaking at room temperature (25º C $\pm$ 2). Immediately after the treatment, the seeds were washed thoroughly in running water to reduce the residual effect of the mutagen on the seed coat. Next , 10 Kr and 0.5 or 1.0 mM of SA; 15 Kr and 1.0 or 5.0 mM of SA; 20 Kr and 0.5 or 1.0 mM of SA; 30 Kr and 1.0 or 5.0 mM of SA were used. The procedure adopted was similar to that used for the individual treatments.

Treated seeds were planted in pots in a glass-house, and 30-day-old seedlings were transplanted in the field in rows. Plants spacing was 15 cm within rows and 80 cm between rows.

Data on seedling growth injury and M_1 spike sterility was recorded on 40 and 50 plants selected at random from each treatment, respectively.

M_2 generation was raised from M_1 spikes, in a glass-house, and frequency of chlorophyll deficiency mutation was scored seven days after germination, based on a M_1 panicle progenie and a M_2 population, for both treated and control populations. The number of mutants by 100 M_2 seedling was also calculated, and, according to Gaul (1960), this proportion has some advantages over other indexes. An average of 31,701 plants per treatment were evaluated.

The factorial combination of treatments, including control was (Table 1):

Number and treatment:
1. control (distilled water)
2. 10 Krad (gamma-rays) and distilled water
3. 15 Krad (gamma-rays) and distilled water
4. 20 Krad (gamma-rays) and distilled water
5. 30 Krad (gamma-rays) and distilled water
6. control (pH 3.0)
7. 15 Krad (gamma -rays) and pH 3.0
8. 30 Krad (gamma-rays) and pH 3.0
9. 0,5 mM of SA and pH 3.0
10. 10 Krad (gamma-rays) and 0.5 mM of SA (pH 3.0)
11. 20 Krad (gamma-rays) and 0.5 mM of SA (pH 3.0)
12. 1,0 mM of SA (pH 3.0)
13. 10 Krad (gamma-rays) and 1.0 mM of SA (pH 3.0)
14. 15 Krad (gamma-rays) and 1.0 mM of SA (pH 3.0)
15. 20 Krad (gamma-rays) and 1.0 mM of SA (pH 3.0)
16. 30 Krad (gamma-rays) and 1.0 mM of SA (pH 3.0)
17. 5,0 mM of SA (pH 3.0)
18. 15 Krad (gamma-rays) and 5.0 mM of SA (pH 3.0)
19. 30 Krad (gamma-rays) and 5.0 mM of SA(pH 3.0)

Table 1 – The factorial combination of gamma -rays and sodium azide tratments, including the control groups.

Gamma-rays	Sodium azide (mM)				
(Krad)	0(pH 7)	0(pH 3)	0.5(pH 3)	1.0(pH 3)	5.0(pH 3)
0	T1	T6	T9	T12	T17
10	T2	- [1/]	T10	T13	-
15	T3	T7	-	T14	T18
20	T4	-	T11	T15	-
30	T5	T8	-	T16	T19

[1/] Not observed.

Dosages and concentrations used in this experiments, the immersion time in the SA solutions, and other methodological aspects were based in Rao (1977), Rao and Rao (1983), Guimarães (1978), Reddi and Rao (1988) e Reddi and Suneeta (1992).

The C^2 test was used to test the homogeneity of the mutation spectra and the additive effect of the combined treatment. In the last case, the expected number was calculated using the sum of the proportional effect of a single treatment.

The frequency of chlorophyll mutations was calculated in the following proportions:

Msp = Total number of mutations x 100 / No. of M_1 panicle progeny = Mutations on M_1 panicle progeny basis

Msd = Total number of mutations x 100 / No. of M_2 plants = Mutations on M_2 plants basis

Mtsd = Total number of mutants x 100 / No. of M_2 plants = Mutants on M_2 plants basis

The mutagenic efficiency was determined by the following proportions:

Msp/I, Msp/L, Msp/S, Msd/I, Msd/L, Msd/S, Mtsd/I, Mtsd/L, Mtsd/L, Mtsd/S

Where:

I = injury = % seedling height reduction
L = % lethality /death rate of M_1 plants till maturity.
S = Fertility reduction percentage (%) as defined by the following formula:

$$S = 100 \left(1 - \frac{\frac{Nsd}{Nsp} \times X}{\frac{Nsd}{Nsp} \times C} \right)$$

where:

Nsd = number of M2 plantules
Nsp = number of M1 panicules

$\frac{Nsd}{Nsp} \times C$ = fertility percentage in the control

$\frac{Nsd}{Nsp} \times X$ = fertility percentage in the m treatment (dosage or concentration)

RESULTS AND DISCUSSION

Table 1 shows the treatment efficiency data. Single treatments tended to be more efficient with the increase in dosage or concentration, but they decreased after reaching a maximum level. Msp higher efficiency levels were reached with the combined treatment of 20 Krads of gamma-rays and 1,0 mM of SA. Higher efficiencies were obtained with SA on an M_1 spike basis and mutants on an M_2 seedling basis. The azide treatment was remarkably higher in producing chlorophyll mutations than the gamma-rays, both on single and combined treatments.

In general, the azide treatment recorded the highest percentage of chlorophyll mutations, followed by the combined treatment and finally by gamma radiation alone. However, when the frequency of mutants in the M_2 plants basis was considered, the combined treatment 15 Kr of gamma-rays + 5 mM of SA was slightly more effective than the best single treatment (5mM of SA). In average, the frequency of chlorophyll mutations was much higher in SA than in gamma-rays.

Data on efficiency by treatments are presented in Table 2. Single treatments tend to increase with the dosages of mutagens, however ,only after reaching a maximum drop/fall. Msp high efficiency levels were reached with 20 Krads to gamma-rays and 0.5 and 1.0 mM to SA. As for the Msd, the maximum efficiency level varied from 10 to 20 Krads, being more sensible to Msd/S and Msd/L concerning the gamma-rays. On the other hand, great concentrations of SA were necessary to reach maximum efficiency varying from 1.0 up to 5.0 mM. In the efficiencies concerning Mtsd, the gamma-rays reached maximum values with 15 to 30 Krads (being Mtsd/L the less sensitive) and SA with 1.0 and 5.0 mM.

However, absolute values for SA efficiency are generally far superior than those for gamma-rays. The combined treatments had intermediary efficiency levels in relation to the single treatments, with some exceptions; i.e. , they increased the efficiency level of the single treatment by gamma-rays and decreased the efficiency level of the single treatment by SA.

By putting together the higher efficiency levels achieved by the considered mutation factor and the different damages/deficiencies, the SA was more efficient than the gamma-rays, on average, when Msp and Msd were used, and it did not differ greatly from Mtsd. As for the Msp and Msd, higher efficiency levels were reached for the L damages and lower for the I . As for the Mtsd, the highest efficiency level was reached by the L damage and the lowest by the S. The higher efficiency observed in lethality is important; however, its practical utility is limited since there is a not very expressive efficiency in sterility, as it happened in this study.

The spectrum and frequency of the chlorophyll mutations in all treatments are presented in Table 3, where the albino type of mutations is predominant in physical and chemical mutagen-treated populations, as well as in the combined treatments with rare exceptions. In the gamma-ray treatments, the frequency of viridis was the next largest frequency after the albino, whereas in chemical treatments and in the combined treatments, some exceptions were recorded. Inequalities in other types of mutations were not very pronounced.

The predominance of the albino type over viridis, in the spectrum that resulted from the treatment with physical and chemical mutagens, is not consistently shown in the literature. Ando (1970), found a larger albino rate using ethylene oxide (EO), Ethyleneimine (EI) and ethyl methane sulfonate (EMS), as well as gamma-rays. Similar results were reached by Miah and Awan (1971) with neutrons and gamma-rays and by Rao (1977) with EMS and gamma-rays. On the other hand, Kaul and Bhan (1977) found a higher albino rate in one of the two varieties studied; nevertheless, in a third variety, a larger proportion of viridis was found. Finally, Rao and Rao (1983) produced higher proportions of albinos by applying methyl methane sulfonate (MMS), N-nitrous-N-methyl urethane (NMU) and hidroxyl amine (HA).

Larger proportions of viridis in rice using AS, were reported by Bhan and Kaul (1976) and Awan et al. (1980);by Reddi and Reddi (1984) using MMS, dms and dES;by Reddi and Rao (1988) using SA in two of the three treated varieties, and by Reddi and Suneetha (1992) using EMS.

Table 2 – Efficiency levels in single and combined treatments with gamma-rays and SA in rice.

Treatment	Msp/I[1/]	Msp/L	Msp/S	Msd/I	Msd/L	Msd/S	Mtsd/I	Mtsd/L	Mtsd/S
Gamma-rays(Kr)									
10	0.52	2.65	0.30	0.007	0.035	0.004	0.024	0.125	0.014
15	-1.71	0.55	0.09	0.034	0.010	0.001	-0.17	0.054	0.009
20	0.68	0.20	0.10	0.013	0.004	0.002	0.080	0.022	0.012
30	0.22	0.18	0.08	0.001	0.005	0.002	0.032	0.025	0.011
SA (mM)									
0.5	0.94	1.19	2.08	0.012	0.015	0.026	0.031	0.039	0.069
1.0	1.02	3.12	1.91	0.019	0.058	0.036	0.058	0.176	0.108
5.0	0.79	1.67	0.81	0.025	0.054	0.026	0.067	0.142	0.068
Gamma-rays (Kr)+ SA (mM)									
10 + 0.5	0.60	0.68	0.40	0.007	.008	0.004	0.039	0.044	0.026
20 + 0.5	0.48	0.65	0.25	0.008	0.010	0.004	0.047	0.064	0.025
10 + 1.0	1.04	1.34	0.58	0.017	0.021	0.009	0.094	0.121	0.052
15 + 1.0	0.39	0.85	0.31	0.011	0.025	0.009	0.046	0.102	0.037
20 + 1.0	0.29	0.47	0.27	0.008	0.013	0.008	0.046	0.075	0.042
30 + 1.0	0.21	0.33	0.20	0.013	0.021	0.013	0.053	0.082	0.052
15 + 5.0	0.34	0.70	0.30	0.019	0.039	0.017	0.069	0.141	0.062
30 + 5.0	0.17	0.20	0.15	0.018	0.021	0.016	0.031	0.038	0.028

[1/] Msp-Mutation frequency on spike basis; Msd-mutation frequency on seedling basis; Mtsd-Mutation frequency on mutant basis; I – The seedling injury (seedling height reduction); L – The M_1 maturity lethality and S – The reduction in M_1 seed fertility.

In other cultures, the predominance of albinos also has conflicting results. Aasveit (1967) found, in barley, a combination of results which favored the development of albinos and viridis in the two types of mutagens in three experiments with physical and chemical mutagens.

The predominance of albinos over viridis in the spectra of chlorophyll mutations was also observed in combined treatments of gamma-rays and EMS or diethylsulpahte (dES) Kaul and Bhan (1977). However, Reddi and Rao (1988) found larger proportions of viridis in the Fujiminori and Jaya varieties with the combined treatment of gamma-rays and AS.

Differences were observed in the spectra of the combined treatments in relation to the respective single treatments (Table 4), but with an exception. Sixteen treatments (50 Krads of gamma-ray and 1.0 mM of SA) did not differ from the spectra in treatment 5 (30 Krad). This suggests that higher dosages of gamma-rays could equal the spectra of the combined treatments, but this happened only in one case out of the eight observed.

Table 3 – Frequency and spectrum of chlorophyll mutations in the treatment by gamma-rays and SA.

Treatment [1/]	Albino	Víridis	Xantha	Tigerina	Striata	Outros	Total
1	0	20	0	0	0	0	20
2	157	65	8	1	0	7	238
3	203	2	4	0	9	1	219
4	140	51	2	3	2	20	218
5	142	37	3	0	1	1	184
6	3	0	0	0	0	4	7
7	267	14	1	0	1	26	309
8	70	19	96	6	20	1	212
9	104	14	21	0	1	10	150
10	277	47	3	0	0	4	331
11	275	88	43	0	6	47	459
12	702	211	130	45	5	102	1195
13	496	113	37	51	13	29	739
14	280	60	127	15	0	138	620
15	293	86	24	25	28	31	587
16	132	17	5	3	0	1	158
17	356	280	88	20	9	14	767
18	128	74	66	49	6	116	439
19	9	2	0	10	0	0	21
Totals	4134	1200	658	228	101	552	6873
Rates	0,6015	0,1746	0,0957	0,0332	0,0146	0,0803	1,00

[1/] Treatments are described in Material and Methods.

Table 4 – Homogeneity c^2 test of spectrum of clorophyll mutations of combined treatment of gamma-rays and SA with the respective single treatment.

Treatments contrast	X^2
T_{10} (T_2:10Kr gamma-rays + T_9:0.5 mM of SA) vs T_2 (10 Kr gamma-rays)	42.64 **
T_{10} (T_2:10Kr gamma-rays + T_9:0.5 mM of SA) vs T_9 (0.5 mM of SA)	52.85 **
T_{11} (T_4:20Kr gamma-rays + T_9:0.5 mM of SA) vs T_9 (0.5 mM of SA)	18.66 **
T_{11} (T_4:20Kr gamma-rays + T_9:0.5 mM of SA) vs T_4 (20Kr gamma-rays)	16.89 **
T_{13} (T_2:10Kr gamma-rays + T_{12}:1.0 mM of SA) vs T_2 (10Kr gamma-rays)	59.31 **
T_{13} (T_2:10Kr gamma-rays + T_{12}:1.0 mM of SA) vs T_{12} (10Kr gamma-rays)	54.78 **
T_{14} (T_3:15Kr gamma-rays + T_{12}:1.0 mM of SA) vs T_3(15Kr gamma-rays)	164.46 **
T_{14} (T_3:15Kr gamma-rays + T_{12}:1.0 mM of SA) vs T_{12}(1.0 mM of SA)	119.61 **
T_{15} (T_4:20Kr gamma-rays + T_{12}:1.0 mM of SA) vs T_4 (20Kr gamma-rays)	26.08 **
T_{15} (T_4:20Kr gamma-rays + T_{12}:1.0 mM of SA) vs T_{12} (1.0 mM of SA)	49.02 **
T_{16} (T_5:30Kr gamma-rays + T_{12}:1.0 mM of SA) vs T_5 (30Kr gamma-rays)	7.34 ^{ns}
T_{16} (T_5:30Kr gamma-rays + T_{12}:1.0 mM of SA) vs T_{12} (1.0 mM of SA)	39.49 **
T_{18} (T_3:15Kr gamma-rays + T_{17}:5.0 mM of SA) vs T_3 (15Kr gamma-rays)	239.42 **
T_{18} (T_3:15Kr gamma-rays + T_{17}:5.0 mM of SA) vs T_3 (5.0 mM of SA)	247.61 **
T_{19} (T_5:30Kr gamma-rays + T_{17}:5.0 mM of SA) vs T_5 (30Kr gamma-rays)	82.43 **
T_{19} (T_5:30Kr gamma-rays + T_{17}:5.0 mM of SA) vs T_{17} (5.0 mM of SA)	86.38 **

Table 5 – Homogeneity χ^2 test of spectrum of chlorophyll mutations (albina and viridis) of the combined treatment of gamma-rays and SA with the respective single treatment.

	Albino		Viridis	
Gamma-rays doses	60.45**		63.03**	
SA concentrations	349.07**		146.57**	
Combined treatments	371.19**		27.72**	
10 Kr + 0.5mM and 10 Kr	24.02**	(+)	15.05**	(-)
10 Kr + 0.5mM and 0.5mM	12.91**	(+)	2.21ns	(=)
20 Kr + 0.5mM and 20 Kr	1.15ns	(=)	134.28**	(-)
20 Kr + 0.5mM and 0.5 mM	4.27*	(+)	7.85**	(+)
10 Kr + 1.0mM and 10 Kr	0.11ns	(=)	2.41ns	(-)
10 Kr + 1.0mM and 1.0mM	13.58**	(+)	1.83ns	(=)
15 Kr + 1.0mM and 15 Kr	143.69**	(-)	18.16**	(+)
15 Kr + 1.0mM and 1.0mM	30.33**	(-)	20.46**	(-)
20 Kr + 1.0mM and 20 Kr	13.09**	(-)	8.60**	(-)
20 Kr + 1.0mM and 1.0mM	12.45**	(-)	2.56ns	(-)
30 Kr + 1.0mM and 3.0 Kr	2.16ns	(+)	5.59*	(-)
30 Kr + 1.0mM and 1.0 mM	36.30**	(+)	4.74*	(-)
15 Kr + 5.0mM and 15 Kr	235.95**	(-)	36.35**	(+)
15 Kr + 5.0mM and 5.0mM	34.61**	(-)	51.98**	(-)
30 Kr + 5.0mM and 30 Kr	11.44**	(-)	1.37ns	(-)
30 Kr + 5.0mM and 5.0mM	0.10ns	(=)	6.48*	(-)

[1/] means that the combined treatment originated more frequency of the albino or viridis type of mutations;

[2/] means that the combined treatment originated less frequency of the albino or viridis type of mutations.

As the main spectra differences were due to the albinos and viridis homogeneity, a test was carried out to analyze the relative proportions of these types of mutants (Table 5). The spectra of albinos and viridis were different in the gamma-ray, SA and combined treatments. However, some interesting aspects were observed in the homogeneity test of each combined treatment regarding the respective single treatment. The combined treatment tended to increase the frequency of albinos in six cases and to decrease in seven cases; but as for the proportions of viridis, the combined treatments tended to decrease the frequency in eleven cases and to increase in three cases. This suggests that the combined treatment tends to diminish viridis frequency.

ACKNOWLEDGMENTS

We thank the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for the financial support.

RESUMO

Eficiência mutagênica da combinação de raios-gama e a azida sódica (NaN_3) em sementes de arroz

Sementes do cultivar de arroz de sequeiro IAC-1246 foram tratadas com dosagens de 10, 15 e 20 e 30 Krad de raios-gama e concentrações de 0,5, 1,0 e 5,0 mM de azida sódica (AS) com o objetivo de estudar e induzir mutantes de clorofila. Os tratamentos combinados incrementaram os danos, principalmente sobre a altura de plântula e fertilidade de panículas M_1.Os tratamentos com AS mostraram maior freqüência de mutações de clorofila , seja em tratamento individual ou em combinação. O espectro de mutações de clorofila proporcionado pelos raios-gama e pela azida sódica foi parecido quanto aos diferentes tipos de mutações, seja em tratamento individual ou combinado. A eficiência da AS foi maior que a de raios-gama em concentrações de de 1,0 e 5,0 mM e menor em 0,5 mM. Quanto ao efeito

aditivo na combinação de mutagênicos, em média, os danos tiveram efeitos aditivos superiores a freqüências de mutações.

REFERENCES

Aasteveit, K. 1967. Effects of combinations of mutagens on mutation frequency in barley p.5-14. In: IAEA.FAO. Mutation in Plant Breeding II. Ed. IAEA, Viena.

Ando, A. 1970. Efeito biológicos de combinação de tratamento com raios gama, cisteina e algumas substancias alquilantes sobre sementes de arroz. Ph.D. Diss. Escola Superior de Agricultura "Luiz de Queiroz"/ USP, Piracicaba, Brazil.

Awan, A.M.; Konzak, C.F.; Rutger, J.N. and Nilan, R.A. 1980. Mutagenic effects of sodium azide in rice. Crop Science. 20:663-668.

Bhan, A.K. and Kaul, M.L.H. 1976. Frequency and espectrum of chlorophyll-deficient mutations in rice after treatment with radiation ans alkylating agents. Mutation Research. 36:311-318.

Fehr, W.R. 1987. Mutation breeding. p.287-297. In: Fehr, W.R. Principles of cultivar development. Macmillan Publishing, New York.

Gaul, H. 1960. Critical analysis of the methods for determining the mutation frequency after seed treatment with mutagens. Genética Agrária. 12: 297-318.

Gaul, H.; Frimel, G.; Gighner, T. and Ulonska, E. 1972. Efficiency of mutagenesis. p.121-139. In: FAO.IAEA. Induced Mutation and Plant Improvemnet. Ed. IAEA/FAO, Londrina.

Guimarães, E.P. 1978. Estudos da sensitividade de sementes de arroz (*Oriza sativa* L.) à radiação gama, nêutrons, sulfato de dietila (DES) e azida sódica. M.S. Thesis. Escola Superior de Agricultura "Luiz de Queiroz"/ USP, Piracicaba, Brazil.

Kaul, M.L.H. and Bhan, A. K. 1977. Mutagenic effectivenes and efficiency of EMS, DES, and Gamma-rays in rice. Theoretical and Applied Genetics. 50:241-246.

Konzak, C.F.; Nilan, R.A,; Wagner, J. and Foster, R.J. 1965. Efficient chemical mutagenesis. p. 49-70. In: IAEA.FAO. The use of induced mutations in plant breeding. Pergamo Press, Roma.

Miah, A.J. and Awan, M.A. 1971. Induced mutations in rice. p.77-89. In: FAO.IAEA. Rice Breeding with induced mutations. Ed. IAEA, Viena.

Montalván, R.M. 1995. Eficiência mutagênica de radiação gama e azida sódica em sementes de arroz (*Oryza sativa* L.). Ph.D. Diss. Escola Superior de Agricultura "Luiz de Queiroz", ESALQ/USP. Piracicaba.

Rao, D.R.M. 1977. Relative effectiveness and efficiency of single and combination tratament ussing gamma-rays and sodiun azide in inducing chlrophyll mutations in rice. Cytologia. 42: 443-450.

Rao, M.G. and Rao, V. M. 1983. Mutagenic efficiency, effec-tiveness and factor of effectiveness of physical and chemical mutagens in rice. Cytologia. 48: 427-436.

Reddi, S. and Reddi, V.R. 1984. Frequency and espectrum of chlorophyll mutants in the seed in rice by chemical mutagens. Theoretical and Applied Genetics. 67: 231-233.

Reddi, T.V.V.S. and Rao, D.R.M. 1988. Relative effectiveness and efficiency of single and combination treatment using Gamma-Rays and Sodium Azide in inducing chlorophyll mutation in rice. Cytologia. 53: 491-98.

Reddi, T.V.V.S. and Suneetha, J. 1992. Chlorophyll-deficient mutations induced in rice by alkylating agents and azide. Cytologia. 57: 285-288.

Steel, R.G.D. and Torre, J.H. 1960. Principles and procedures of statistics. 2.ed. Mc Graw-Hill, New York.

Received: January 30, 2001;
Accepted: August 02, 2001.