

Original Research Article

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Induced Macromutational Spectrum and Frequency of Viable Mutants in M₂ Generation of Rice (*Oryza sativa* L.)

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ABSTRACT

The present study was conducted to know the frequency and spectrum of viable mutations after irradiation and revealed that viable mutation frequency was high on M₁ plant basis than M₂ seedling basis in gamma rays, electron beam and recurrent treatment. Electron beam gave higher frequency of viable mutants than gamma rays. The spectrum of viable mutants included a total of 286 mutants of gamma rays, 516 in electron beam and 153 mutants in recurrent treatment dose in ADT - 37. In ADT - 45, a total of 235 mutants in gamma irradiated population and 455 mutants in electron beam treated population and 159 mutants recurrent treatment dose were observed. Economic mutants such as plants possessing earliness, lodging resistance, grain size and high yielding mutants were isolated in the present study. A wide spectrum of viable mutants was observed in electron beam than gamma rays in both the genotypes used. The variety ADT - 37 had more number of viable mutants than ADT - 45. Electron beam was found to be effective than Gamma rays in inducing chlorophyll and viable mutants.

Keywords

Viable mutants,
Electron beam,
Gamma rays,
Recurrent mutation.

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Introduction

Rice (*Oryza sativa* L.) which belongs to the family Poaceae, is the life and the prince among cereals as this unique grain helps to sustain two thirds of the world's population. Improvement in rice yield and quality beyond the benefits of the green revolution of 30 years ago are required to meet the demands of increasing global population. Crop improvement in rice is mainly carried out through, conventional hybridization and selection procedures, ideotype breeding, heterosis breeding, wide hybridization, marker assisted breeding and genetic engineering. Conventional breeding methods

such as pedigree, bulk, and back cross breeding with some modifications are the principal procedures followed in the improvement of self-pollinated crops. The usefulness of these methods is limited because of its several drawbacks; limited parent participation, low genetic variability, reduced recombination and rapid fixation of genes. In recent years, research in mutation breeding is in progress in different cereals. Among the cereals, rice is the important crop being grown in most regions of India and enjoys a place of importance as a food crop.

Induced mutation can rapidly create variability in quantitatively and qualitatively inherited traits in crops (Maluszynski *et al.*, 2005 and Muduli and Mishra, 2007). These mutations provide beneficial variation for practical plant breeding purpose. The mutagens may cause genetic changes in an organism, break the linkage and produce many new promising traits for the improvement of crop plants (Shah *et al.*, 2008).

Viable mutations include those affecting the morphology of different parts of the plants such as habit, stature, leaf, stem, pod and seed. Wide spectrum of viable morphological mutations was isolated in M₂ generation (Wani, 2011). Mutations are phenotypically classified into two groups (Gaul, 1964); macro mutations: These are easily detectable in individual plants, phenotypically visible and morphologically distinct and they are qualitatively inherited genetic changes, and occur in major genes or oligogenes; and micro mutations: These result in a small effect that, in general, can be detected only by help of statistical methods and quantitatively inherited genetic changes, and occur in minor genes or polygenes. Recurrent treatment during subsequent generations has been proposed to create additional variability over a single treatment (Briggs and Constantin, 1977). Since the induced mutations has been accepted as a useful tool in plant breeding, a systematic study of characterization of gamma rays, electron beam and recurrent dose induced mutants in M₂ generation of rice (*Oryza sativa* L.) was made in the present study.

Materials and Methods

$$\text{MF based on } M_1 \text{ plant basis (\%)} = \frac{\text{Number of viable mutant } M_1 \text{ families}}{\text{Total number of } M_1 \text{ families}} \times 100$$

Plant material

The seeds M₂ of two varieties namely ADT 37 and ADT 45 for the induction of mutation treatment were obtained from Department of Plant Breeding and Genetics, Agricultural College and Research Institute, Madurai, India. The experiment was conducted during Kharif 2015 at Agricultural College and Research Institute, TNAU, Madurai.

Recurrent treatment with Gamma rays

From M₁ generation, 200 seeds of Gamma rays and Electron beam were treated again with gamma rays as recurrent treatments. The different doses Gamma rays (100Gy, 200Gy), electron beam (200Gy, 300Gy), and recurrent treatment (200Gy + 100Gy) constituted the materials for this study. The irradiated seeds were raised in the nursery; 21 days old seedlings were transplanted into main field. The recommended packages of practices were followed throughout the period of crop growth.

Frequency and spectrum of viable mutants

The frequency and spectrum of different types of viable mutants were scored at various developmental stages of M₂ plants particularly from flowering to maturity period. These mutants were classified for deviation from the normal looking plants and taking into consideration the most conspicuous characters namely, stature, duration, leaf shape, grain size etc. The frequency and spectrum of viable mutants were calculated on M₁ plant basis and M₂ seedling basis.

$$\text{MF based on } M_2 \text{ seedling basis (\%)} = \frac{\text{Number of viable mutant } M_2 \text{ seedlings}}{\text{Total number of } M_2 \text{ seedlings}} \times 100$$

Results and Discussion

Gaul (1964) classified the viable mutations as macro and micro mutations, while Swaminathan (1964) grouped them as viable mutations, macro mutations and systemic mutations. In general, any mutational event may bring large or small change in the phenotype. Such changes in macro mutants have the highest significance in plant breeding because they may sometimes give a desired phenotype. A number of new commercial varieties have been originated from induced macro mutants and they proved their usefulness in attaining distinct breeding objectives. It is also possible to induce new features, which do not exist in the available range of variability in a well adapted and high yielding variety. Frequency of such mutations also serves as an index of mutagenic sensitivity of various mutagenic agents and their dose effects (Waghmare and Mehra, 2001).

The viable mutations isolated in this study showed that, the changes in major traits which could be utilized in future breeding programme where reshuffling of traits may be tried by conventional breeding methods. However, from the previous studies it was concluded that most of the morphological mutants identified in M_2 generation failed to inherit in M_3 generation. According to the statements of Luo *et al.*, (2012), these characters may be controlled by recessive genes or susceptible to environment. Moreover, whatever the changes occurred in the plants due to mutation was an error according to the plants geometry. They tend to rectify it in due course through recombinational events. That is why most of the observed mutants were not inherited in future generations. Thus, evolving a new

phenotype with consistent expression through mutation is a chance event rather than a choice.

Frequency of viable mutants

The frequency of viable mutants ranged from 30.77 to 42.31 on M_1 plant, and 3.07 to 4.52 on M_2 plant bases in ADT 37 following gamma rays treatment. The viable mutants were observed in all the doses. The maximum and minimum frequencies were observed in two doses 200 Gy and 100 Gy. In electron beam treatment, the frequency ranged from 65.38 to 76.92, 6.30 to 7.35 on M_1 plant and M_2 plant bases respectively. The maximum and minimum frequencies were observed in two doses of 300 Gy and 200 Gy. In ADT 45, the frequency of viable mutants ranged from 34.62 to 46.15 on M_1 plant and 2.65 to 3.54 on M_2 plant bases. The maximum and minimum frequencies were observed in two bases 300 Gy and 200 Gy of gamma rays. The frequency of electron beam varied from 50.00 to 61.54 on M_1 plant and 5.33 to 6.76 M_2 plant bases. The maximum frequency was obtained at 300 Gy on M_1 plant basis and 300 Gy on M_2 plant basis respectively. In the present study, viable mutation frequency was high on M_1 plant basis than M_2 seedling basis in gamma rays, electron beam and recurrent treatment (Table 1; Figs. 1 and 2).

Spectrum of viable mutants

The viable mutants were scored in M_2 generation based on their phenotypic changes in qualitative attributes. The viable mutants were grouped based on variability in plant type, stem colour, leaf type, duration, tillering habit, panicle type, lodging resistant, grain type and other characters (Table 2).

A total of 286 gamma ray mutants, 516 electron beam mutants and 153 electron beam + gamma rays mutants were identified in ADT 37. In ADT 45, there were 235 gamma ray mutants and 455 electron beam mutants and 159 electron beam + gamma rays mutants were identified.

Tall mutants were observed in higher proportion after electron beam treatment in both varieties though the tall mutants had less number of productive tillers they recorded higher grain and straw yield, and matured earlier by 10 days. Sharma (1985) reported similar tall variety.

In the present study, mutants with well exerted panicles were also identified in both the varieties with low productive tillers but with more number of filled grains. Hence these mutants offer scope for further study to improve the yield. However mutation affecting duration, plant type, panicle type, leaf modifications, tillering habit, panicle variation, lodging resistance and grain size were observed in both the varieties among the duration groups, early and late maturing mutants were identified. The early mutants were in higher proportion than late mutants in gamma ray for ADT - 45.

The early mutants were maximum in proportion with electron beam treatments in both the varieties they recorded more number of productive tillers, high single plant yield and increased plant height. They are economical to utilize in the further study because of its high yield and lodging resistant. However the late maturing mutants showed relatively high productive tiller and recorded increased single plant yield. Such early and late mutants had been identified by Mahadevappa *et al.*, (1983), Hakim *et al.*, (1985) and Sharma (1985) in rice.

Grain mutants were higher in the electron beam treated population than gamma rays

treated population. A mutant with medium slender grain was recorded in ADT – 37 rice. Even though it recorded high single plant yield (39.40 g) than its control by the virtue of its consumer preference this mutants offer very good scope for further study to improve yield potential, under cultivated condition. However, awned mutants observed in the same genotype recorded low single plant yield. Nodal mutant, boat leaf, broad flag leaf, low tillering, purple grain colour, beaked grain and partially awned grain type mutant in ADT - 37 and broad leaf, boat leaf, panicle exertion, enclosed panicle exertion type, purple grain colour, purple coloured stigma in ADT - 45 were higher in gamma rays followed by electron beam than recurrent dose.

ADT – 45 variety is highly susceptible to lodging and hence the need for dwarf mutants from these varieties with high yielding potential was felt much. Viable mutants for plant type *viz.*, early/late flowering, dwarf type, high tillering habit, narrow rolled leaf, upper albino, lodging resistant, grassy and extreme dwarf and lanky culms were observed in M₂ population of ADT - 45. The present mutation breeding has developed dwarf mutants in higher number with electron beam treatments when compared to gamma rays (Sree Rangasamy and Anandakumar (1983). The reduction in plant height would be the desirable attributes for nonlodging plant type. The dwarf mutant isolated also showed increased yield over control, in the present investigation. Exposure of rice seeds to 20 Krad and 30 K rad gamma rays decreased the culm length thereby increasing the lodging resistance (Futsuhara *et al.*, 1967).

Dwarfness was mostly caused by recessive major gene mutations (Mikaelsen, 1980). Shadakshari *et al.*, (2001) reported a higher frequency of dwarf/semi-dwarf non-lodging mutants in five rice varieties treated with gamma rays.

Table.1 Viable mutation frequencies in M₂ generation ADT 37 and ADT 45

ADT 37							ADT 45					
Mutagens (Dose)	No. of M ₁ plants		No. of M ₂ seedlings		Mutation frequency (%)		No. of M ₁ plants		No. of M ₂ seedlings		Mutation frequency (%)	
	Plants forwarded	Segregating	Studied	Chlorophyll mutants	M ₁ plant basis	M ₂ seedling basis	Plants forwarded	Segregating	Studied	Chlorophyll mutants	M ₁ plant basis	M ₂ seedling basis
Gamma rays												
Control	10	-	943	-	-	-	10	-	976	-	-	-
100 Gy	26	8	3873	119	30.77	3.07	26	9	3843	102	34.62	2.65
200 Gy	26	11	3691	167	42.31	4.52	26	12	3756	133	46.15	3.54
Electron Beam												
Control	10	-	923	-	-	-	10	-	964	-	-	-
200 Gy	26	17	3844	242	65.38	6.30	26	13	3826	204	50.00	5.33
300 Gy	26	20	3726	274	76.92	7.35	26	16	3712	251	61.54	6.76
Recurrent dose (Electron beam + Gamma rays)												
200 Gy +100 Gy	14	12	2043	153	85.71	7.49	14	10	2087	159	71.43	7.62

Table.2 Spectrum of viable mutations in M₂ generation of ADT 37 and ADT-45

S.No.	Viable mutants	ADT-37						ADT-45					
		Spectrum			Percentage			Spectrum			Percentage		
		GR	EB	R	GR	EB	R	GR	EB	R	GR	EB	R
1	Plant type												
a	Tall	17	26	8	5.94	5.04	5.23	11	25	9	4.68	5.49	5.66
b	Dwarf	14	34	9	4.90	6.59	5.88	7	18	6	2.98	3.96	3.77
e	Grassy and extreme dwarf mutant	11	27	7	3.85	5.23	4.58	5	22	8	2.13	4.84	5.03
2	Stem colour												
a	Dark green	25	20	6	8.74	3.88	3.92	10	16	6	4.26	3.52	3.77
b	Light green	16	16	5	5.59	3.10	3.27	8	18	7	3.40	3.96	4.40
3	Leaf												
a	Broad leaf	9	13	4	3.15	2.52	2.61	12	17	8	5.11	3.74	5.03
b	Narrow leaf	11	18	5	3.85	3.49	3.27	6	21	4	2.55	4.62	2.52
e	Boat leaf	5	8	2	1.75	1.55	1.31	8	6	2	3.40	1.32	1.26
f	Leaf sheath anthocyanin colouration	-	-	-	-	-	-	3	5	2	1.28	1.10	1.26
4	Flag leaf												
a	narrow leaf	5	13	6	1.75	2.52	3.92	14	5	6	5.96	1.10	3.77
b	broad leaf	13	18	5	4.55	3.49	3.27	7	11	4	2.98	2.42	2.52
5	Duration												
a	Early	12	24	8	4.20	4.65	5.23	13	14	4	5.53	3.08	2.52
b	Late	20	31	10	6.99	6.01	6.54	16	18	6	6.81	3.96	3.77
6	Tilering habit												
a	High	12	25	4	4.20	4.84	2.61	8	18	4	3.40	3.96	2.52
b	Low	10	15	4	3.50	2.91	2.61	9	20	3	3.83	4.40	1.89
7	Panicle type (length in cm)												
a	Very short (<16cm)	8	16	3	2.80	3.10	1.96	9	18	3	3.83	3.96	1.89
b	Long (26-30 cm)	6	12	4	2.10	2.33	2.61	10	21	4	4.26	4.62	2.52
c	Very long (>30 cm)	7	15	3	2.45	2.91	1.96	10	28	4	4.26	6.15	2.52

8	Panicle exertion												
a	partially exerted	3	9	5	1.05	1.74	3.27	8	8	2	3.40	1.76	1.26
b	Enclosed	4	13	4	1.40	2.52	2.61	9	10	2	3.83	2.20	1.26
9	Secondary branching in panicle	3	11	3	1.05	2.13	1.96	4	8	3	1.70	1.76	1.89
10	Pigmented node	0	9	3	0.00	1.74	1.96	-	-	-	-	-	-
12	Vivipary mutants	7	10	3	2.45	1.94	1.96	3	5	1	1.28	1.10	0.63
13	Nodal mutant	0	7	5	0.00	1.36	3.27	0	8	2	0.00	1.76	1.26
14	Grain colour												
a	Purple	6	7	2	2.10	1.36	1.31	5	6	2	2.13	1.32	1.26
b	Black	5	8	5	1.75	1.55	3.27	3	5	1	1.28	1.10	0.63
15	Purple coloured stigma	6	5	2	2.10	0.97	1.31	0	2	1	0.00	0.44	0.63
16	Grain type												
a	Long slender	3	7	2	1.05	1.36	1.31	4	12	3	1.70	2.64	1.89
b	Long bold	8	15	3	2.80	2.91	1.96	2	6	4	0.85	1.32	2.52
c	Medium slender	5	13	4	1.75	2.52	2.61	4	8	2	1.70	1.76	1.26
d	Triple grain mutant	0	17	1	0.00	3.29	0.65	0	5	5	0.00	1.10	3.14
e	Beaked grain	5	9	4	1.75	1.74	2.61	3	9	5	1.28	1.98	3.14
17	Awned grains												
a	Partially awned	13	21	6	4.55	4.07	3.92	7	19	9	2.98	4.18	5.66
b	Completely awned	17	24	8	5.94	4.65	5.23	10	20	11	4.26	4.40	6.92
18	Lodging resistant	-	-	-	-	-	-	7	23	16	2.98	5.05	10.06
	Total	286	516	153	100.00	100.00	100.00	235	455	159	100.00	100.00	100.00

Fig.1 Mutation frequency of viable M₁ plant basis –M₂ generation

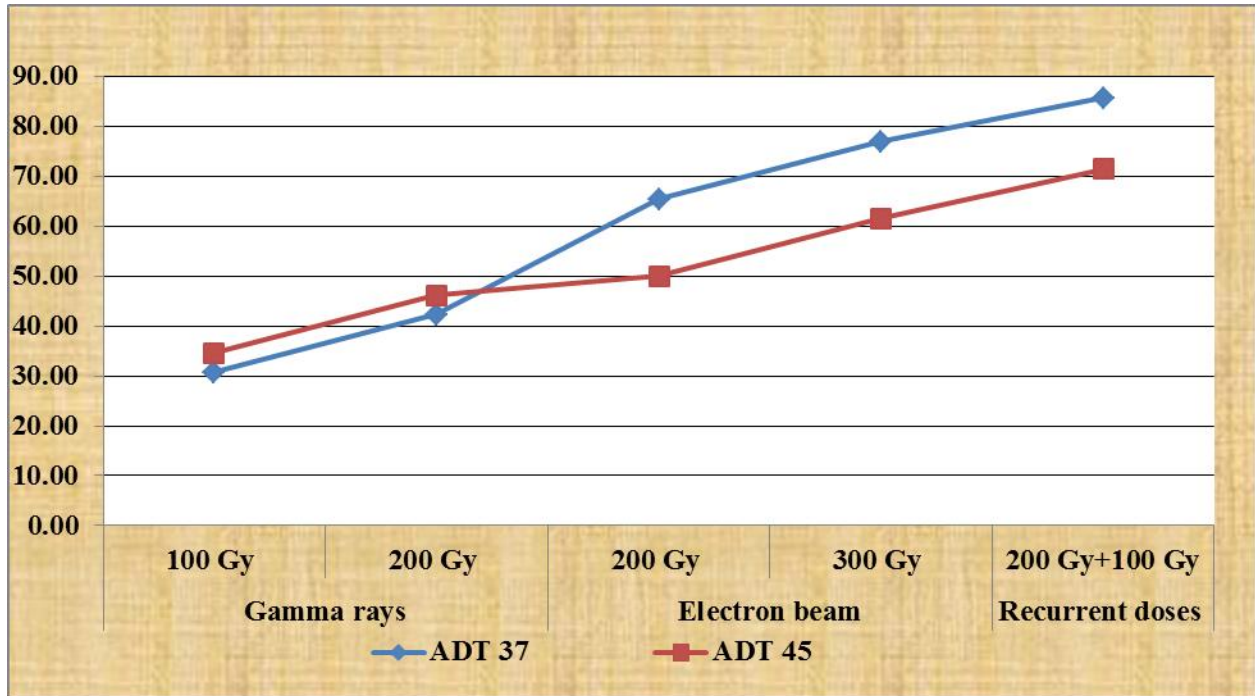
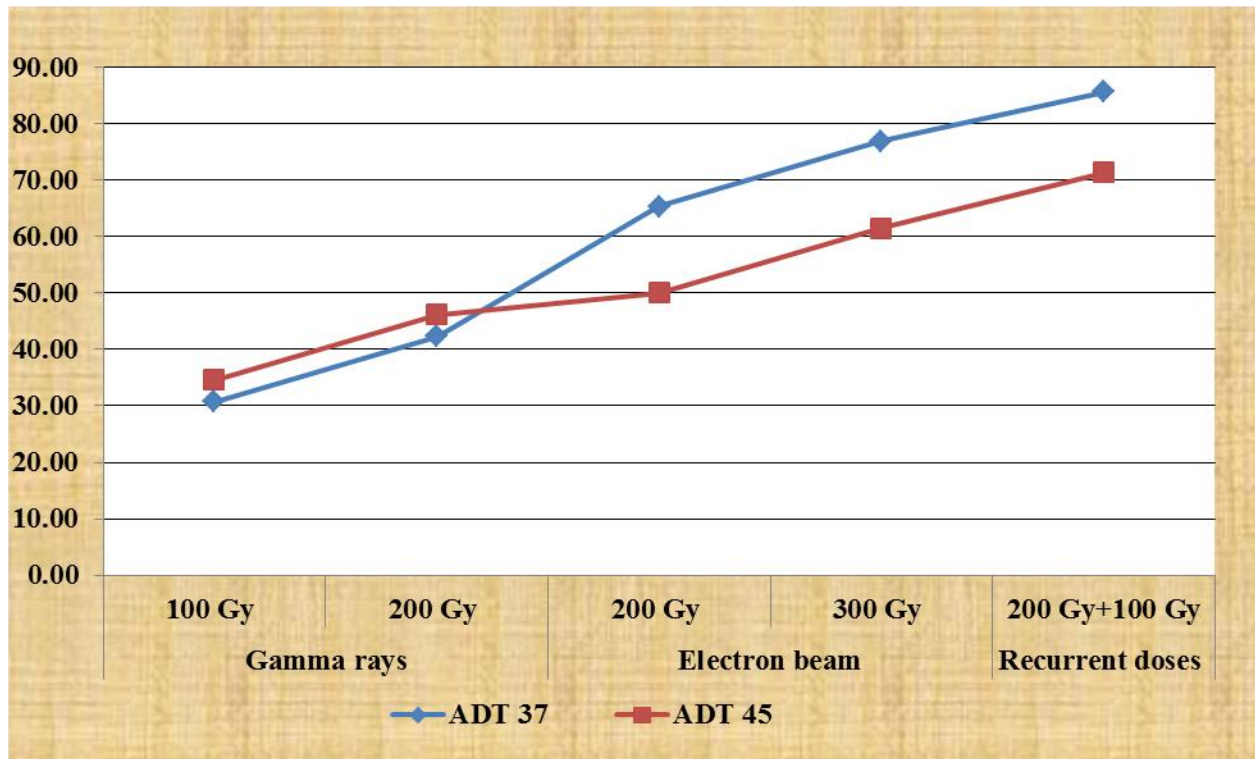


Fig.2 Mutation frequency of viable M₂ plant basis –M₂ generation



They also isolated early flowering and high yielding mutants from these varieties. Frequency of semi dwarf mutants was high in lower doses of gamma radiation when compared to higher doses in gamma irradiated populations of ADT - 37. Shadakshari *et al.*, (2001) reported the occurrence of higher frequency of dwarf/semi-dwarf non-lodging early flowering and high yielding mutants in five rice varieties treated with gamma rays. Anitha Vasline (2013) conducted an experiment in rice to study the effect of the chemical mutagen EMS in rice varieties ADT 43 and IR 64. In M₂ generation, the widest spectrum of viable mutants such as early, spreading, narrow leaf, lax panicle, tall and viviparous mutant were noticed.

Few of the grain type mutants with varying grain size and shape gave low grain yield. These observations showed pleiotropic gene action on the traits. Gaul *et al.*, (1968) and Reddy and (Reddy and Reddy 1974) also made similar observations in barley and rice respectively. Micke (1999) viewed that pleiotropy is a typical attribute of induced mutations. Functioning of such gene could be explained through crosses (Gaul *et al.*, 1968). The mutational changes in gene functioning act as regulators for several other genes. Mutations affecting pleiotropic genes governing several characters were also reported by Deshmukh *et al.*, (1972). According to Blixt (1972), morphological changes are either due to pleiotropic gene action or of cryptic chromosomal deletions.

Fineness of grains to a considerable extent determines the acceptability and market value of rice. Grain dimension (length and breadth) shows high heritability (Chandraratna, 1964), and least influenced by environmental variations. Studies of different workers (Jalil and Yamaguchi, 1964; Reddy and Reddy, 1974) indicated that grain shape and size are controlled by more than one gene and may be

altered in both positive and negative directions to different degrees.

In the present study, four different combinations of grain type's viz., (i) long slender (ii) long bold (iii) long bold and (iv) medium slender were found which indicated that length and shape of grain in rice are independently inherited characters and can be combined through mutational manipulation. Simultaneous changes in flowering time, plant height, number of productive tillers per plant, panicle length and single plant yield, grain shape and size as observed suggested the existence of pleiotropic gene action, which is in much agreement with the earlier contention of Reddy and Reddy (1974). More number of viable mutants was obtained in ADT 37. Electron beam was found to be superior in missing viable mutants than gamma rays.

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