



Research Note

Assessment of chlorophyll and viable mutation in M₂ generation of rice (*Oryza sativa* L.)

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Abstract:

The present investigation was carried out to study the mutagenic effect of the chemical mutagen, EMS on two well known short duration rice varieties viz., ADT 43 and ADT 45 of Tamil Nadu, South India. A wide spectrum of chlorophyll mutants namely albino, xantha and chlorina were observed in all the treatments in M₂ generation. The albino type were most frequent and chlorina were least common. The treatments produced the relative frequency and wide spectrum of viable mutants like early, spreading, narrow leaf, lax panicle, hairy grain, awned grain and grain length mutants in M₂ generation. Early mutants were observed maximum at 0.8 per cent EMS in ADT 43, while spreading mutants were observed maximum at 0.8 per cent EMS in ADT 45. Maximum narrow leaf mutants were observed at 0.6% EMS in ADT 43.

Key Words: Rice; Chlorophyll mutations; viable mutations

Mutation breeding has been widely used for the improvement of plant characters in various crops . It is a powerful and effective tool in the hands of plant breeders especially for autogamous crops having narrow genetic base (Micke, 1988). Mutagenic agents have been used to induce useful phenotypic variations in plants for more than seventy decades (Anitha Vasline *et al.* 2005). During the past 70 years, more than two thousand five hundred and forty three mutant cultivars from 175 plant species including cereals, oilseeds, pulses, vegetables, fruits, fibers and ornamentals have been officially released in 50 countries all over the world (Maluszynski *et al.* 2000; Chopra, 2005). In any mutation breeding programme, selection of an effective and efficient mutagen is very essential to produce high frequency of desirable mutation. Many chemical mutagens have been employed for obtaining useful mutants in various crop species (Sanjeev Singh and Singh, 2003). Among the chemical mutagens, ethyl methane sulfonate (EMS) induces a vastly higher proportion of point mutations (Minocha and Arnason, 1962; Hajra, 1979).

Chlorophyll mutants are employed as markers for the evaluation of gene action of mutagenic factors in inducing mutation studies (Gaul, 1964). The spectrum and frequency of chlorophyll mutants are assessed in M₂ population easily and is being used as primary index of effectiveness of mutagens and mutability of the genotypes towards the mutagens which in turn would be useful to generate the wide array of desirable mutants in the treated population. In the present study attempt has been made to understand the comparative response of the chemical mutagen EMS on two popular rice varieties of Tamil Nadu viz., ADT 43 and ADT 45 with respect to the frequency and spectrum of chlorophyll and viable mutants in rice.

Two popular rice genotypes ADT 43 and ADT 45 of Tamil Nadu were chosen to study the effect of the chemical mutagen. The characteristic features of both the rice varieties are presented in Table-1. The dose at which 50 per cent reduction in seed germination was taken as LD₅₀ value. Based on LD₅₀ value, the

treatment doses 0.4, 0.6 and 0.8 per cent were chosen to conduct the field experiments. Three sets of 200 well dried seeds were selected and soaked in double distilled water for 12 hours. Then they were treated with 0.4, 0.6 and 0.8 per cent concentration of EMS for four hours under controlled conditions. After the treatment, the seeds were thoroughly washed with running tap water for eight to ten times. Pre-soaked seeds in distilled water for 16 hours were used as the control. The M_1 generation was raised during December, 2006. The seeds subjected to EMS at different doses were sown along with control in three meter rows with a spacing of 15 cm between plants and 30 cm between rows. In each treatment, a total number of 200 seeds were sown. The experiment was laid out in Randomized Block Design with two replications. The recommended cultural operations and need based plant protections were carried out.

The M_2 Subscript generation was grown from single plant M_1 Subscript progeny seeds. Ten randomly selected M_1 plants per treatment along with control were tagged and harvested individually and advanced to M_2 generation. They were raised during April, 2007. The treated and control populations were observed thoroughly in M_2 Subscript generation for lethal and non-lethal mutations from emergence till the age of four weeks after germination and were registered.

The M_2 plants at seedling stage were screened upto 15 days to record the various chlorophyll mutants periodically. The different types of chlorophyll mutants were scored separately for calculating the spectrum of mutants. Also, some viable mutants in comparison to control were scored and described in respect of altered characters from the normal plants.

Frequency and spectrum of chlorophyll mutations.

Five different types of chlorophyll mutants were obtained when seedlings were 8-20 days old. The spectrum of chlorophyll mutations included: albina, chlorina, and xantha in both the varieties. Chlorina type survived up to maturity while the others died at seedling stage.

The varieties ADT 43 and ADT 45 showed varied influences for EMS treatment. In M_1 generation, both the varieties showed reduction in seed germination and seedling survival in the different treatments. Chlorophyll deficient seedlings were counted and recorded as chlorophyll mutants in M_2 generation. A wide spectrum of chlorophyll mutants were observed in the mutagenic treatments. Albino (white colour leaves) and xantha (Yellow colour

leaves) died at the early stage of growth. The chlorina mutants had pale green leaves which gradually turned normal green. The chlorina type was viable and reproduced later. The chlorina mutants were observed at 0.8 per cent EMS in ADT 43 were found to be low. This may be due to zygotic inviability. Similar results were expressed by Singh and Sanjeev Singh (2001) and Vennila (2005).

In the M_2 generation, some viable mutants were also screened. All the viable changes in comparison to control were scored and described in respect of altered characters from the normal plants. The frequencies of morphological deviants in respect to duration, leaf size, panicle type and seed size were described as viable mutants.

Gaul (1964) classified viable mutants as micro and macro mutations. A mutational event may be accompanied by a larger or smaller change in phenotype. Such changes have the highest significance in plant breeding and have been stressed by Brock (1970) and Sigubjornsson (1972). All characters which are of interest to plant breeders can be either altered or amended by mutation.

In the present investigation, wide spectrum of viable mutants were observed in the mutagenic treatments. The spectrum of viable mutants were early, spreading, narrow leaf, lax panicle, long grain, awned grain and hairy grain (Table 3).

Early mutants were observed maximum at 0.8 per cent EMS in ADT 43, while spreading mutants were observed maximum at 0.8 per cent EMS in ADT 45. Spreading mutants had wider angle among tillers and require more spacing. Maximum narrow leaf mutants were observed at 0.6% EMS in ADT 43. Lax panicle type was observed in all the treatments with maximum at 0.8% EMS in ADT 43. Similar results were observed by Vennila (2005). Long grain mutants were observed maximum at 0.8 per cent EMS in ADT 43. Awns were seen on the slender grains more frequently. They were thick and like long hairs. These awned mutants were observed maximum at 0.8 per cent EMS in ADT 43. Small hairs were seen on the slender grains. These mutants were seen maximum at 0.8 per cent EMS in ADT 43.

The treatment at 0.8 per cent EMS in ADT 43 produced a higher frequency of viable mutation (17.42%) followed by 0.8% EMS in ADT 45 (3.61%). The lowest frequency of viable mutation was produced by 0.4% EMS in ADT 45 (0.91%) (Table 4). Comparison of the two varieties ADT 43



and ADT 45, for different doses of EMS, revealed that 0.8% EMS was the most effective dose in inducing a wide range of viable mutants in rice.

On the overall basis, the albino type chlorophyll mutations were most frequent and chlorina were least common. Several unique and interesting mutations were induced in this study. In our study, the frequency of chlorophyll mutation was enhanced with increasing dose. Similar result was also reported by Sheeba et al. (2004). The fertile mutants generated in this study could be valuable for linkage and mapping studies. Further more, these mutants can also be used to isolate genes involved at different developmental stages of plants. This reveals that mutation breeding is a valid and effective crop breeding method in rice.

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Table 1. Characteristic features of the rice varieties selected for treatment

Characters	ADT 43	ADT 45
Parentage	IR 50 x I.X. Ponnai	IR 50 x ADT 37
Photosensitivity	Insensitive	Insensitive
Habit	Semidwarf	Semidwarf
Duration	110 days	110 days
Grain traits	MS	MS
100 grain weight	1.55g	1.75g
Special characters	Resistant to green leaf hopper and tolerant to salinity	Moderately resistant to brown plant hopper and moderately resistant to sheath blight.

Table 2. Spectrum of chlorophyll mutants in M₂ generation

Treatment (Conc.)	Albino	Xantha	Chlorina	Total
ADT 43				
0.4% EMS	2	1	1	4
0.6% EMS	4	3	2	9
0.8% EMS	7	5	4	16
Total	12	9	7	29
ADT 45				
0.4% EMS	1	1	-	2
0.6% EMS	3	4	3	10
0.8% EMS	5	3	2	10
Total	9	8	5	22



Table 3. Spectrum of viable mutants in M₂ generation

Treatment (Conc.)	Early	Spreading	Narrow leaf	Lax panicle	Long grain	Awned grain	Hairy grain	Total
ADT 43								
0.4% EMS	1	1	2	2	-	-	2	8
0.6% EMS	6	3	4	4	2	1	5	25
0.8% EMS	10	2	1	7	26	32	30	108
Total	17	6	7	13	28	33	37	141
ADT 45								
0.4% EMS	-	-	2	-	2	-	-	4
0.6% EMS	2	2	2	5	4	-	-	15
0.8% EMS	4	5	1	3	6	1	2	22
Total	6	7	5	8	12	1	2	42

Table 4. Frequency of viable mutants in M₂ generation Verify totals , entire table and rewrite

Treatment (Conc.)	Total plants studied	Viable mutants observed	Mutation frequency (%)
ADT 43			
0.4% EMS	420	-	-
0.6% EMS	480	8	1.66
0.8% EMS	510	25	4.90
Total	620	108	17.42
ADT 45			
0.4% EMS	410	-	-
0.6% EMS	440	4	0.91
0.8% EMS	480	15	3.12
Total	610	22	3.61