

Biological Effects and Chlorophyll Mutations Induced by NG. Ragi*

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Two ragi varieties viz., MS. 2698 (Bihar) and Sarada (Andhrapradesh) were treated with N-methyl-N-nitroso-N'-nitroguanidine. The germination, survival and seedling height at transplanting got reduced as the dose of the mutagen increased in both the varieties. MS. 2698 showed a higher percentage of sterility than Sarada consequent on the chemical treatment. The LD 50 based on survival was around 10 mM for MS. 2698 and 5 mM for Sarada. The chlorophyll mutation frequency estimated on M₂ seedling basis was much less than that estimated on M₁ spike basis. Sarada recorded greater mutation frequency than MS. 2698. There was a varietal difference in the spectrum of mutation also. The variety Sarada recorded *albina* types besides *xantha* and *viridis* types. A higher percentage of *tigrina* was also observed in Sarada as compared to MS. 2698. The mutagenic effectiveness and efficiency were conferred by the same dose 5 mM for two hours of chemical soaking.

The aim of induced mutagenesis is on one hand, from a theoretical stand point, to uncover the action mechanisms of mutagens and on the other hand, from a practical stand point, to establish efficient methods of inducing mutations for plant breeding (Kawai, 1969). The latter objective was kept in mind in pursuing the present investigation for inducing variability through chemical mutagenesis in ragi (*Eleusine coracana* (L.) Gaertn.). The mutagenic effects of N-methyl-N-nitroso-N'-Nitroguanidine (NG), not having been studied so far in ragi, have not been studied in two ragi varieties and the results are reported.

MATERIAL AND METHODS

Two ragi varieties MS. 2698 of Bihar and Sarada of Andhra Pradesh were chosen for the study. For each

dose of the mutagen, one hundred well-filled seeds of each variety with a uniform moisture content of 10.5 per cent were used. The seeds were presoaked in distilled water for eight hours prior to the mutagenic treatment.

N-methyl-N-nitroso-N'-nitroguanidine (NG), a chemical mutagen in solid form, with a molecular weight of 147.09 g was used. Appropriate quantities of the mutagen were dissolved in double distilled water to get concentrations of 0, 2.5, 5.0, 7.5, 10.0 and 12.5 mM. The seeds were treated with the said doses for two hours and four hours. The seeds were sown in pots, which were given suitable growing conditions and on 21st day seedlings were transplanted in the main field.

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TABLE 1 Effect of mutagen on M₁ generation (Percentage on respective controls)

| Treatment | MS 2698 | | | | Sarada | | | |
|-----------------------------|------------------------|-----------------------|---------------------------|--------------------------|------------------------|------------------------|---------------------------|--------------------------|
| | Germination | Survival | Seedling height reduction | Seed fertility reduction | Germination | Survival | Seedling height reduction | Seed fertility reduction |
| Two hours chemical soaking | | | | | | | | |
| Control | 100.0 | 100.0 | 0 | 0 | 100.0 | 100.0 | 0 | 0 |
| NG 2.5 mM | 81.6 | 81.8 | 25.57 | 3.80 | 75.5 | 77.5 | 22.82 | 0.37 |
| NG 5.0 mM | 77.6 | 68.1 | 40.46 | 5.87 | 65.3 | 65.0 | 27.34 | 3.06 |
| NG 7.5 mM | 65.3 | 61.3 | 41.22 | 5.90 | 61.2 | 60.0 | 30.80 | — |
| NG 10.0 mM | 65.3 | 56.8 | 55.73 | 3.90 | 59.2 | 55.0 | 32.87 | 3.44 |
| NG 12.5 mM | 61.2 | 38.6 | 63.36 | 12.84 | 44.9 | 45.0 | 41.87 | 1.17 |
| Four hours chemical soaking | | | | | | | | |
| Control | 100.0 | 100.0 | 0 | 0 | 100.0 | 100.0 | 0 | 0 |
| NG 2.5 mM | 82.0 | 72.0 | 35.47 | 6.24 | 84.0 | 77.2 | 22.41 | 0.03 |
| NG 5.0 mM | 74.0 | 62.7 | 47.17 | 11.93 | 66.0 | 56.8 | 31.03 | 4.64 |
| NG 7.5 mM | 70.0 | 60.4 | 50.19 | 11.34 | 52.0 | 50.0 | 38.79 | 8.55 |
| NG 10.0 mM | 66.0 | 48.8 | 59.25 | 17.05 | 48.0 | 37.6 | 43.97 | 9.03 |
| NG 12.5 mM | 52.0 | 44.1 | 61.51 | 22.06 | 52.0 | 36.3 | 54.74 | 13.02 |
| Period of soaking | NS (SE=0.78) | NS (SE=0.60) | NS (SE=0.06) | NS (SE=0.55) | NS (SE=0.67) | NS (SE=0.33) | ** (SE=0.17) (CD=0.53) | NS (SE=0.78) |
| Treatments | ** (SE=0.78) (CD=2.43) | * (SE=0.73) (CD=2.27) | ** (SE=0.07) (CD=0.22) | ** (SE=0.67) (CD=2.18) | ** (SE=2.08) (CD=8.53) | ** (SE=0.41) (CD=1.28) | ** (SE=0.21) (CD=0.65) | NS (SE=0.96) |
| Periods X treatments | NS (SE=1.18) | NS (SE=1.04) | ** (SE=0.10) (CD=0.31) | NS (SE=0.96) | NS (SE=0.95) | NS (SE=0.58) | NS (SE=0.30) | NS (SE=1.31) |

* : Significant at 5 per cent level.
 ** : Significant at 1 per cent level
 NS : Not significant
 SE : Standard error
 CD : Critical difference

The mutagenic effects in M1 generation were studied in terms of germination, survival seedling height at transplanting and seed fertility. The seeds collected from first formed three tillers of each M1 plant were advanced to M2 as spike progenies. The two genotypes were studied in two separate trials in the adjacent plots of the same field in randomised blocks design with three replications. The M1 spikes and M2 plants segregating for chlorophyll mutations were scored and classified according to the system proposed by Gustafsson (1940). The mutagenic effectiveness and efficiency were estimated by the method of Konzak *et al.* (1965).

RESULTS AND DISCUSSION

(a) M1 effects

The effects of the mutagen on M1 generation was measured in terms of germination, survival, seedling height and seed fertility (Table I). In both the varieties the treatments differed significantly in their effects. There was a general reduction in germination and survival in both the varieties as the dose of the mutagen increased. Similar observations have been made by Sreekantaradhya (1971) in his studies with EMS treatments in ragi. There was a significant reduction in the seedling height in the M1 generation. The magnitude of reduction increased with the increasing dose of the mutagen and the results of the

present study are in conformity with the earlier reports of Goud *et al.* (1969). Although the seed fertility was not appreciably affected by the dose of the mutagen, there was a differential response due to genotypes for seed fertility, evidenced by the higher percentage of sterility in MS. 2698 ranging from 0.46 to 11.67 than in Sarada. Though this aspect has not been studied in ragi earlier, Gafoor Arain (1974) noticed genotypic differences for fertility reduction in barley.

The LD 50 based on survival was around 10 mM for MS. 2698 and around 5 mM for Sarada. The surviving plants from the LD 50 doses under both the levels of soaking were advanced to M2 for studying the chlorophyll mutations.

(b) Chlorophyll mutations

A total number 19,358 M2 plants from 240 M1 spikes in MS. 2698 and 17,241 from 143 spikes in Sarada was studied for the estimation of chlorophyll mutation frequency (Table II).

The frequency of chlorophyll mutations estimated on M1 spikes was much higher than that estimated on M2 seedling basis in all the treatments in both the varieties. There was a definite differential response by the two genotypes studied, Sarada recording a greater frequency of chlorophyll mutations than MS. 2698.

TABLE II (a) Frequency of chlorophyll mutations in M₂ generation.

| Treatment | M ₁ (Spike) family basis | | | M ₂ Seedling basis | | |
|------------------|-------------------------------------|----------------------------|----------------------------|-------------------------------|----------------------------|-----------------------------|
| | No. of families studied | No. of families segregated | Mutations per 100 families | No. of seedlings studied | No. of seedling segregated | Mutations per 100 seedlings |
| MS. 2698 | | | | | | |
| Control | 30 | — | — | 2872 | — | — |
| NG 5 mM (2 hrs) | 60 | 31 | 51.66 | 5760 | 41 | 0.711 |
| NG 10 mM (2 hrs) | 46 | 16 | 34.78 | 4180 | 19 | 0.454 |
| NG 5 mM (4 hrs) | 77 | 19 | 24.67 | 4078 | 24 | 0.588 |
| NG 10 mM (4 hrs) | 27 | 14 | 51.85 | 2468 | 20 | 0.810 |
| Sarada | | | | | | |
| Control | 36 | — | — | 4444 | — | — |
| NG 5 mM (2 hrs) | 39 | 21 | 53.85 | 3797 | 30 | 0.790 |
| NG 10 mM (2 hrs) | 22 | 11 | 50.00 | 2322 | 18 | 0.775 |
| NG 5 mM (4 hrs) | 25 | 17 | 68.00 | 4591 | 40 | 0.871 |
| NG 10 mM (4 hrs) | 21 | 12 | 57.14 | 2087 | 26 | 1.245 |

(c) Spectrum of mutations

Xantha type of mutation was the most predominant in the variety MS. 2698 while Sarada produced more of

viridis than the other types (Table III). *Albina* type was totally absent in MS. 2698 whereas in Sarada, a low frequency of this type was observed. Sarada recorded *tigrina* mutations in

TABLE II (b) Spectrum of Chlorophyll Mutations

| Treatment | Albina | | Xantha | | Viridis | | strita | | Tigrina | | Others (Complex) | |
|------------------|--------|-------|--------|-------|---------|-------|--------|-------|---------|-------|------------------|-------|
| | No | % | No | % | No | % | No | % | No | % | No | % |
| MS 2698 | | | | | | | | | | | | |
| NG 5 mM (2 hrs) | — | — | 14 | 34.15 | — | — | 1 | 2.44 | — | — | 26 | 63.41 |
| NG 10 mM (2 hrs) | — | — | 4 | 21.05 | 2 | 10.52 | 1 | 5.26 | — | — | 12 | 63.17 |
| NG 5 mM (4 hrs) | — | — | 9 | 37.50 | — | — | 1 | 4.17 | — | — | 14 | 58.33 |
| NG 10 mM (4 hrs) | — | — | 6 | 30.00 | — | — | 1 | 5.00 | 2 | 10.00 | 11 | 55.00 |
| Sarada | | | | | | | | | | | | |
| NG 5mM (2 hrs) | 1 | 3.33 | 5 | 16.67 | 11 | 36.67 | 3 | 10.00 | 2 | 6.67 | 8 | 26.66 |
| NG 10 Mm (2 hrs) | — | — | 1 | 5.56 | 2 | 11.11 | 7 | 38.89 | 2 | 11.11 | 6 | 33.33 |
| NG 5 mM (4 hrs) | 1 | 2.50 | 6 | 15.00 | 12 | 30.00 | 3 | 7.50 | 2 | 5.00 | 16 | 40.00 |
| NG 10 mM (4 hrs) | 3 | 11.54 | 5 | 19.23 | 14 | 15.38 | 1 | 3.85 | 2 | 7.69 | 11 | 42.31 |

all the treatments while in MS. 2698, it was observed only in the highest dose. Thus, there was a clear difference in the spectrum of mutation between the two genotypes studied. Besides the above five types of mutations, complex types such as albo-xantha, alboviridis and virido-xantha have also been observed to occur in reasonably high frequencies indicating the effectiveness of the chemical. It will be relevant to add here that Swaminathan *et al.* (1968) observed that NG was a very efficient mutagen in inducing mutations in wheat. There was a higher percentage of seed sterility in the variety MS. 2698 than in Sarada in the M₁ generation. This variety also yielded more number of *xantha* type of mutations. This relationship between *xantha* and sterility has been reported by Sreekantaradhy (1971) in ragi.

(d) Effectiveness and efficiency

The effectiveness and the efficiency of the mutagen were calculated with reference to chlorophyll mutations (Gaul *et al.*, 1972). In both the varieties chosen for the investigation, the highest mutagenic effectiveness was observed in the lowest dose 5 mM at two hours of soaking (Table IV).

The effectiveness was found to be much less in the higher dose (10 mM) and/or with longer soaking period. Apparently, lower concentrations cause less lethality and this enables the biological material to express more induced mutations. Mutagenic efficiency was higher in the dose 5mM for two hours when estimated on the basis of lethality, injury or sterility in both the varieties. Although, the most effective mutagen need not be the most efficient

TABLE III Mutagenic Effectiveness and Efficiency in M₂ Generation

| Treatments | Survival reduction Percentage (L) | Height reduction percentage (I) | Seed ferti- lity reduc- tion percentage (S) | Mutations per 100M ₂ seedlings (M) | Effective ness Mx100 tc | Efficiency | | |
|------------------|--|--|---|--|-------------------------------|--------------|--------------|--------------|
| | | | | | | Mx100 (L) | Mx100 (I) | Mx100 (S) |
| MS 2698 | | | | | | | | |
| NG 5 mM (2 hrs) | 32.88 | 40.46 | 3.80 | 0.71 | 7.10 | 2.16 | 1.76 | 18.68 |
| NG 10 mM (2hrs) | 43.18 | 50.73 | 3.59 | 0.45 | 2.25 | 1.04 | 0.81 | 12.54 |
| NG 5 mM (4 hrs) | 37.21 | 47.17 | 11.93 | 0.59 | 2.95 | 1.59 | 1.25 | 4.95 |
| NG 10 mM (4 hrs) | 51.16 | 59.25 | 17.05 | 0.81 | 2.03 | 1.58 | 1.37 | 4.75 |
| Sarada | | | | | | | | |
| NG 5 mM (2 hrs) | 35.00 | 27.34 | 3.06 | 0.79 | 7.90 | 2.26 | 2.89 | 25.82 |
| NG 10 mM (2 hrs) | 45.00 | 32.87 | 3.44 | 0.78 | 3.90 | 1.73 | 2.37 | 22.68 |
| NG 5 mM (4 hrs) | 43.18 | 31.03 | 4.64 | 0.87 | 4.35 | 2.02 | 2.80 | 18.75 |
| NG 10 mM (4 hrs) | 61.36 | 43.97 | 9.02 | 1.25 | 3.13 | 2.04 | 2.84 | 13.86 |

Note: t: duration of treatment C: concentration

one (Konzak *et al.* 1965), in the present investigation, the mutagenic effectiveness and efficiency were conferred by the same dose.

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