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Research Article

# Mutagenic effectiveness and efficiency of sodium azide in rice varieties

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**Abstract**

This study was taken up to assess the chlorophyll spectrum induced by Sodium Azide mutagen in two rice cultivars- BPT 2231 and CO 51. The mutagenic effectiveness and efficiency of Sodium Azide across the two genotypes were also observed. The chlorophyll spectrum observed in $M_2$ generation of BPT 2231 is consisted of albino, xantha, chlorina, striata and xantha viridis. In CO 51, albino and xantha occupied the entire chlorophyll spectrum. Albino was predominant in both the varieties with 1.2 per cent in BPT 2231 and 1.15 per cent in CO 51 at 1mM concentration. The mutagenic frequency was higher in BPT 2231 (7.8) than CO 51 (4.0). Considering the efficiency of a mutagen based on lethality, seedling injury and pollen sterility, BPT 2231 recorded higher values 2.79, 1.10 and 0.26 respectively. Like mutagenic frequency, the mutation rate of Sodium Azide was higher in BPT 2231(0.90) than CO 51 (0.40).

**Keywords**

Rice, Mutagenic effectiveness, Mutagenic efficiency, Sodium Azide, Mutation Breeding

## INTRODUCTION

Rice (*Oryza sativa L.),* is an important member of poaceae, the highly appreciable position in world's food chain and its consumption is expected to increase by 67 Mt in 2028 (OECD/FAO-2019). For sustaining the food production and ensuring nutritional security, constant crop improvement is inevitable. The high selection pressure applied so far in rice breeding since, domestication has ended up in decline of genetic variability. Genetic improvement of the crop depends on the sustainment of the variability in the population. The alternative solution to increase genetic variability is induced mutagenesis and it plays a vital role in self-pollinated crop where there is limited variability. Understanding the mutations and their practical utility will help in the elucidation of genetic, physiological and biochemical basis of rice traits. Because of its small genome size, it facilitates the release of higher quality sequences and also due to synteny among the poaceae, other crops do receive benefit from the findings of rice crop (Viana *et al*., 2019).

In induced mutagenesis, selection of mutagen is the most important step and several things are considered for finding out the most effective and efficient mutagen. Two different aspects namely, mutagenic effectiveness and efficiency decide the utility of mutagen. Mutagenic effectiveness measures the mutations induced per unit dose of mutagen. Mutagenic efficiency gives the information on genetic damage in relation to the biological damage caused in $M_1$ generation. The frequency of desirable mutations relies upon both the effectiveness and efficiency of mutagen (Konzak *et al.,* 1965, Nilan *et al*., 1965). Also, Gustafsson, (1951) quoted that chlorophyll mutations are the most convenient method for evaluating the genetic effect of mutation in plants. Considering all these aspects, the present investigation was taken up to assess the impact of different Azide concentrations in $M_1$ and $M_2$ generation of BPT 2231 and CO 51 rice genotypes.

## MATERIALS AND METHODS

The two rice varieties CO 51 and BPT 2231 were collected from Department of Rice, Coimbatore and Rice Research Unit, Bapatla respectively. CO 51 is a short duration (105-110 days), high yielding semi-dwarf rice variety with medium slender grain. BPT 2231 differs from CO 51 with respect to duration alone as it is a long duration rice variety (150-155 days) and it is a major lacuna of BPT 2231. This experiment was taken up to study the effect of mutagen on duration of varieties and also to assess the response of long duration and short duration varieties to the mutagen. The seeds of each variety were tested for optimum moisture content (12%) and then they were treated with Sodium Azide mutagen at eight different concentrations- 1mM, 2mM, 3mM, 4mM, 5mM, 6mM, 7mM and 8Mm. The seeds taken for treatment were hydrated for the period of 20 hours to enhance the mutagenic effect. Five hundred seeds were used for each concentration. The acidic pH of the buffer solution plays a key role in the mutagenic effect of Sodium Azide. Hence, the pH of the phosphate buffer should be 3. Stock solution of Sodium Azide was prepared in 0.1 M phosphate buffer and according to the concentration required, they were added to the seeds placed in graduated centrifuge tubes. The seeds were placed in Orbiter shaker for 4 hours to make sure that the seeds are uniformly exposed to the mutagen. After treatment, the seeds were cleansed with pure water for five times at the time interval of four minutes. The same process is repeated using running water to eliminate the chemical residues. The seeds soaked in buffer were taken as control. The seeds in each concentration were divided into two batches- 200 seeds were used for germination test using roll paper method and remaining seeds were raised in nursery.

Evaluation of $M_1$ generation was carried out for both the varieties. Invitro studies were done to assess the impact of different concentrations on seed germination and lethality effects. The seed germination was taken on seventh day and the seeds with radicle emergence beyond 2 mm were considered as germinated ones. Seedling injury was deduced using shoot length (cm) and root length (cm) on fourteenth day.

For determining the impact of mutagen on pollen fertility status, the unopened flowers were collected from ten randomly selected plants in each treatment including control. The pollen grains were retained in glass slide by gently tapping the stamen excluding the debris. Potassium iodide stain was used for visualizing the pollen status and 1% potassium iodide stain is prepared by dissolving 1g of iodine along with 2g of potassium iodide in 100ml of distilled water. The glass slide was viewed using compound light microscope and the images were recorded with Camera- H1C and Scope image 9.0.1 software. Pollen fertility study, based on Raj and Virmani, (1988) was done as follows: fully stained pollen were fertile ones and sterile pollens were marked by partial staining, malformed and void nature. The plants exhibiting both types of pollen were classified as partially sterile. The pollen fertility status was obtained using the formula,

$$Pollen\ fertility\ percent = \frac{Number\ of\ round\ well\ stained\ pollens}{Total\ number\ of\ pollens\ observed} * 100$$

In BPT2231, 41 $M_2$ families and in CO 51, 49 $M_2$ families were forwarded to $M_2$ generation. The $M_1$ plants were harvested and primary panicle of each plant was raised in panicle to progeny row method. The visual observation of chlorophyll mutants was carried out on 14$^{th}$ day from the time of germination. The characterization of mutants was done based on the classification given by Gustaffson (1940). The distinct chlorophyll mutations observed in $M_2$ rice seedlings were –

Albino- The entire seedling lacks chlorophyll pigment and appears with white leaves. Due to the lacuna of chlorophyll apparatus, it doesn't survive beyond 10-12 days after germination.
Chlorina -The leaves are light green in colour and most of them decline within 20 days. Few vigorous seedlings will manage to complete their lifecycle.

Xantha - Bright yellow colour of leaves and their survival range from 10 – 20 days.
Striata - Green and yellow – white longitudinally striped leaves and they are viable.
Xantha-viridis - Seedlings are characterized by both viridine green colour and bright yellow colour occurring in same leaf.

The chlorophyll mutation frequency was calculated based on $M_2$ seedling basis. Mutagenic effectiveness gives the measure of the frequency of mutations induced by a unit dose of mutagen and efficiency gives the info on biological damage. The formula put forth by Konzak *et al*., (1965) was adapted for calculating mutagenic effectiveness and efficiency based on the mutation frequency of chlorophyll mutants obtained.

$$Mutagenic\ effectiveness = \frac{Mutagenic\ Frequency}{Dose\ (or)\ Concentration * Time}\ Mutagenic\ efficiency = \frac{Mutagenic\ Frequency}{Biological\ Damge}$$

$$Mutagenic\ efficiency = \frac{Mf}{L}, \frac{Mf}{I}, \frac{Mf}{S}$$

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Where,
L- Percent of Lethality in $M_1$ Generation
I-Percent of Seedling injury in $M_1$ Generation
S-Percent of Pollen Sterility in $M_1$ Generation
Mutation rate was found using the formula

$$\text{Mutation rate} = \frac{\text{Sum of values of efficiency (or) effeciveness of particular mutagen}}{\text{Number of treatments of a particular mutagen}}$$

This helps in finding mutations induced by a particular mutagen without the aid of concentration or dose.

## RESULTS AND DISCUSSION

Mutation research mainly focuses on enriching the mutation frequency and manipulation of the mutation spectrum in a desirable manner. The evaluation of chlorophyll spectrum in $M_2$ generation is the most reliable method for assessing the mutagen induced genetic alterations in target plant genotype (Chaturvedi and Singh, 1990). Also, the chlorophyll spectrum studies aid in understanding the potency of mutagen. The spectrum of chlorophyll mutants was diverse in BPT 2231, consisting of albino, xantha, chlorina, striata and xantha viridis (**Fig. 1**.). Classes of chlorophyll mutants observed in CO 51 were albino and xantha. Similar, spectrum of chlorophyll mutants induced by Sodium Azide were reported by Ando *et al.,* (2001) and Nilan *et al.,* (1967). Here, the varietal differences were evident as same mutagen induced different spectrum of chlorophyll mutants in two genotypes and similar events for genotypic difference in rice was reported by Reddi and Rao, (1988). Several genes responsible for chlorophyll biosynthesis are located near centromere and proximal segment of chromosome. The mutagenic effects on these genes result in chlorophyll mutations exhibiting different spectrum in subsequent generations (Goud, 1967 and Swaminathan, 1964). For gaining better insight of nature of mutation, chlorophyll mutants can be isolated and characterized at molecular level.

**Albino** **Xantha-viridis** **Chlorina**

**Xantha** **Striata**

**Fig.1. Chlorophyll spectrum in BPT 2231 and CO 51 induced by Sodium Azide Mutagenesis in $M_2$ generation**

In both the genotypes, the distribution of albino was predominant-BPT 2231(2.7) and CO 51 (3.91) (**Table 1 &2**). Occurrence of higher frequency of albino among chlorophyll mutations in aromatic rice varieties was also put forth by Ando *et al.,* (2001) and Chakravarti *et al.,* (2013). The order of spectrum of chlorophyll mutants in BPT 2231 is Xantha (2.8) > Albino (2.7) >Xantha-viridis (1.8) > Striata (0.4) > Chlorina (0.1). The spectrum of chlorophyll mutants in CO 51 is Albino (3.9) > Xantha (0.04). The values ranged from 0.9-3 in BPT 2231 and 0.33 to 1.16 in CO 51.

Considering concentration wise in two cultivars, 1mM is known to induce higher frequency of chlorophyll mutants (**Fig. 2**.). The dose independent relationship was found between mutagenic frequency and increasing concentrations and no linear trend was observed. General perspective is that the total mutation frequency and spectrum are strongly associated with the dose of mutagen. The trend found here is in accordance with the Gaul (1964), Yamaguchi *et al.,* (2009) where there is no relationship between the dose of mutagen and the mutation spectrum.

**Table 1. Frequency and spectrum of chlorophyll mutants in M2 generation of BPT2231**

| Mutagen | Classes of Chlorophyll Mutants | | | | | Number of Chlorophyll Mutants | Number of Plants Observed | Relative Percentage of Chlorophyll Mutants | | | | | Mutagenic Frequency |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| SA | Albino | Xantha | Chlorina | Striata | Xantha-viridis | | | Albino | Xantha | Chlorina | Striata | Xantha viridis | |
| 1mM | 21 | 20 | 1 | 4 | 8 | 54 | 1808 | 1.2 | 1.1 | 0.1 | 0.2 | 0.4 | 3.0 |
| 2mM | 5 | 2 | | | 6 | 13 | 1512 | 0.3 | 0.1 | 0.0 | 0.0 | 0.4 | 0.9 |
| 3mM | 4 | 7 | | | | 11 | 679 | 0.6 | 1.0 | 0.0 | 0.0 | 0.0 | 1.6 |
| 4mM | 2 | 3 | | | 6 | 11 | 743 | 0.3 | 0.4 | 0.0 | 0.0 | 0.8 | 1.5 |
| 5mM | 2 | 1 | | 1 | 1 | 5 | 587 | 0.3 | 0.2 | 0.0 | 0.2 | 0.2 | 0.9 |
| | | | | | | | | 2.7 | 2.8 | 0.1 | 0.4 | 1.8 | 7.8 |

**Table 2. Frequency and spectrum of chlorophyll mutants in M2 generation of CO 51**

| Mutagen | Classes of Chlorophyll Mutants | | Number of Chlorophyll Mutants | Number of Plants Observed | Relative Percentage of Chlorophyll Mutants | | Mutagenic Frequency |
|---|---|---|---|---|---|---|---|
| SA | Albino | Xantha | | | Albino | Xantha | |
| 1mM | 6 | 4 | 10 | 521 | 1.15 | 0.01 | 1.16 |
| 2mM | 2 | 7 | 9 | 623 | 0.32 | 0.01 | 0.33 |
| 3mM | 6 | 4 | 10 | 680 | 0.88 | 0.01 | 0.89 |
| 4mM | 4 | 1 | 5 | 428 | 0.93 | 0.00 | 0.94 |
| 5mM | 8 | 7 | 15 | 1280 | 0.63 | 0.01 | 0.63 |
| | | | | | 3.91 | 0.04 | 3.95 |

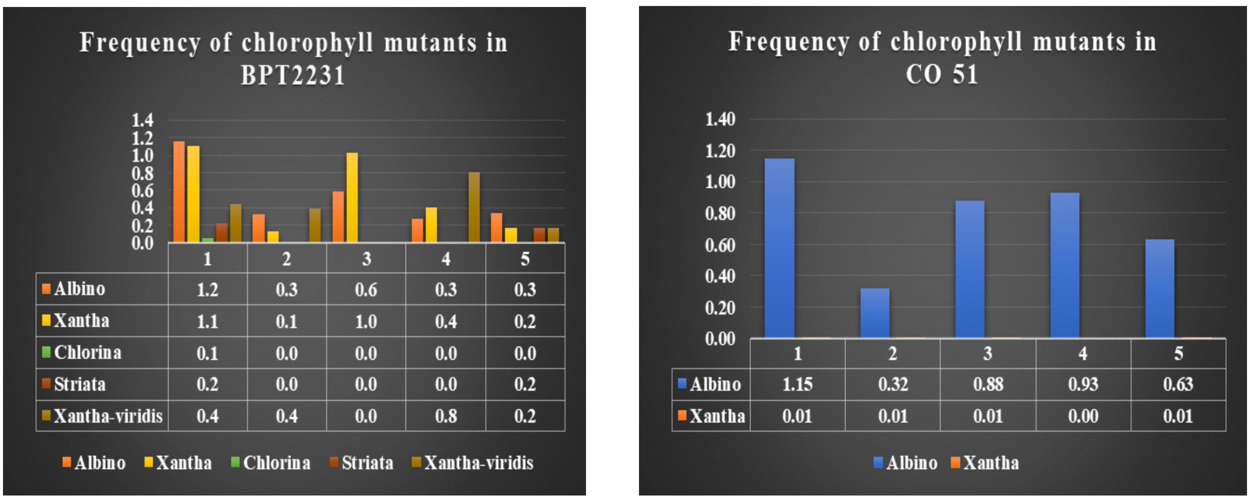


**Fig.2.Frequency of Different Classes of Chlorophyll Mutants in $M_2$ Generation of BPT 2231 and CO 51 Treated with Sodium Azide**

Mutagenic effectiveness indicates the frequency of mutations induced by a unit dose of mutagen and mutagenic efficiency is a measure of the proportion of mutation in relation to undesirable changes like lethality, pollen sterility, spikelet sterility and seedling injury. Three criteria, lethality, seedling injury and pollen sterility were considered for assessing the mutagenic effectiveness and efficiency. Dose dependent linear relationship was observed as the lethality per cent, pollen sterility per cent and seedling injury per cent increased with increase in Azide concentrations. The trend in this case is concomitant with the reports of Awan *et al.,* (1980). The efficiency values varied based on the criteria taken for consideration in both the varieties. The highest effectiveness was found at 1mM concentration in BPT 2231(3.0) and at 1mM in CO 51 (1.16). In BPT 2231(**Table 3**), the efficiency calculated based on lethality recorded the highest value (2.72) at 1mM followed by pollen sterility (0.91) and seedling injury (0.14) at the same concentration. Like BPT 2231, in CO 51 **(Table 4)**, the efficiency was highest at 1mM concentration for lethality (0.12), pollen sterility (0.09) and seedling injury (0.07).

**Table 3. Mutagenic effectiveness and efficiency based on chlorophyll mutations in M2 generation of BPT2231**

| Mutagen | Biological Damage | | | Mutation Frequency | Effectiveness | Efficiency | | |
|---|---|---|---|---|---|---|---|---|
| | Lethality% | % Pollen Sterility | Seedling Injury% | | | Msf/L | Msf/S | Msf/I |
| 1mM | 1.10 | 3.30 | 20.66 | 2.99 | 3 | 2.72 | 0.91 | 0.14 |
| 2mM | 40.00 | 12.70 | 30.52 | 0.86 | 0.43 | 0.02 | 0.07 | 0.03 |
| 3mM | 70.50 | 18.80 | 39.04 | 1.62 | 0.54 | 0.02 | 0.09 | 0.04 |
| 4mM | 84.20 | 54.90 | 53.38 | 1.48 | 0.37 | 0.02 | 0.03 | 0.03 |
| 5mM | 91.60 | 85.40 | 57.87 | 0.85 | 0.17 | 0.01 | 0.01 | 0.01 |
| | | | | 7.80 | 4.5 | 2.79 | 1.10 | 0.26 |

**Table 4. Mutagenic effectiveness and efficiency based on chlorophyll mutations in M2 generation of CO 51**

| Mutagen | Biological Damage | | | Mutation Frequency | Effectiveness | Efficiency | | |
|---|---|---|---|---|---|---|---|---|
| | Lethality% | % Pollen Sterility | Seedling Injury % | | | Msf/L | Msf/S | Msf/I |
| 1mM | 9.50 | 13.30 | 17.65 | 1.16 | 1.16 | 0.12 | 0.09 | 0.07 |
| 2mM | 31.60 | 18.40 | 29.86 | 0.33 | 0.17 | 0.01 | 0.02 | 0.01 |
| 3mM | 59.00 | 22.00 | 42.53 | 0.89 | 0.30 | 0.02 | 0.04 | 0.02 |
| 4mM | 83.60 | 33.90 | 50.68 | 0.94 | 0.23 | 0.01 | 0.03 | 0.02 |
| 5mM | 84.20 | 48.30 | 51.13 | 0.63 | 0.13 | 0.01 | 0.01 | 0.01 |
| | | | | 3.95 | 1.98 | 0.17 | 0.19 | 0.13 |

Lower concentrations of the mutagen proved to have higher effectiveness and efficiency than the moderate and higher concentrations. It was also observed that, the mutagenic efficiency was lowest at 5mM concentration in both the varieties. Similar results were observed in case of mutagenic effectiveness too. The dose dependent decline of efficiency and effectiveness was reported by Ambavane *et al.,* (2015). Mutation rate was higher in BPT 2231 (0.9) than CO 51 (0.40) in terms of effectiveness**(Table 4 and Fig. 3.)**. Also, the mutation rate in terms of efficiency was recorded highest in BPT 2231 (0.56) which was estimated based on lethality criteria**(Table 5 and Fig. 3.)**.

Several factors like genetic base of the genotypes, stage of cell cycle, physiological condition of seed material and nature of mutagen decides the effectiveness and efficiency of the mutagens. Both effectiveness and efficiency of mutagen must be taken into account for recovering the high frequency of desirable mutants (Konzak *et al.,* 1965, Nilan *et al.,*1965). Here, the chlorophyll spectrum in BPT2231 was more diverse than CO 51. Taking mutagenic effectiveness and mutagenic efficiency, BPT 2231 outperformed CO 51 in all three criteria (Lethality, Seedling injury and Pollen sterility). The main aim of this study is to identify the early duration mutants in BPT 2231 and identification of other useful mutants in both the rice varieties. From overall perspective, the results revealed that the response of BPT 2231 to Azide treatments is comparatively appreciable than CO 51.

**Table 5. Mutagen Rate in Terms of Effectiveness and Efficiency in M2 Generation of BPT2231 and CO 51**

| Mutagen | Mutation Rate in Terms of Effectiveness | | Mutation Rate in Terms of Efficiency | | | | | |
|---|---|---|---|---|---|---|---|---|
| | BPT2231 | CO 51 | | BPT2231 | | | CO 51 | |
| Sodium Azide | 0.9 | 0.40 | 0.56 | 0.22 | 0.05 | 0.03 | 0.04 | 0.03 |

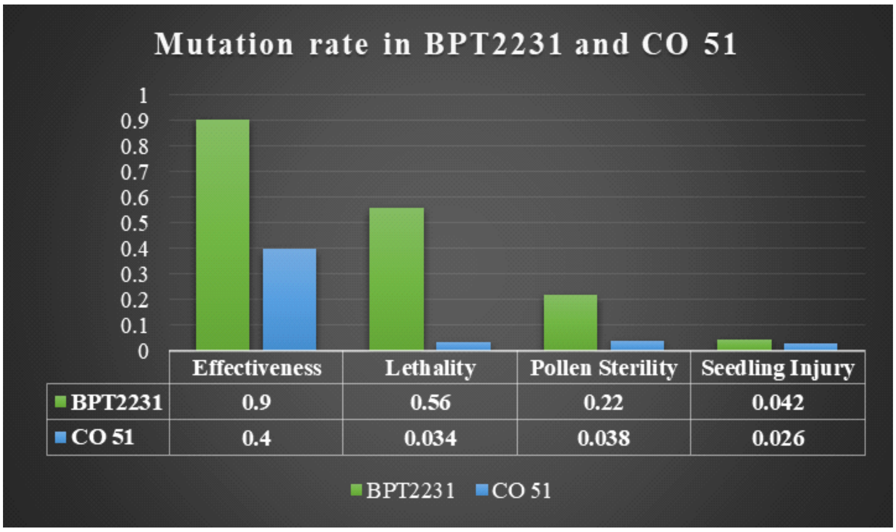


**Fig.3.Mutation rates in terms of effectiveness and efficiency in BPT 2231 and CO 51 treated with sodium azide**

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