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# Physiological and morphological effect of ethyl methanesulfonate on two rice varieties (*Oryza sativa* L.) of Assam

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**Abstract**
Rice is produced in a wide range of location and under a variety of climatic conditions. Many local varieties are available. Genetic variation is required for crop improvement of the useful traits. Mutagenic agents can induced mutations and generate genetic variation. The present investigation aims at finding out the mutagenic effects of the mutagen, Ethyl methanesulfonate in different traits selected such as in germination, seedling height and number of rootlets, root length and number of tillers in the seeds of two indigenous rice varieties of Assam, Ahu and Baismuthi. The experiments were conducted for both laboratory as well as field conditions. The results shows that the highest % germination as observed on the control samples as expected when compared to that of treatment. With increasing EMS amounts there has been a decrease in seedling height. Under field conditions, the plant height (in Ahu) observed during the late stages of seedlings and early stages of adult plants shows that it increase steadily with increase in concentration of EMS in comparison to that of the control whereas the number of root length and roots (in Baismuthi) shows a decrease in increasing EMS concentration in comparison to the control. The number of tillers were also found to decrease with increasing concentration in both the varieties.

**Keywords:** ethyl methanesulfonate, rice, germination, baismuthi, ahu

## Introduction
Rice (*Oryza sativa*) is one of the staple food used by more than half of the world's population because of its significant portion of dietary intake. Rice provides minerals, proteins, vitamins and fibre, although all constituents except carbohydrates are reduced by milling. India is the world's 2nd largest producer of rice and is the largest exporter of rice in the world market. Rice feeds more than 60 percent population of India. Due to its nutritional value and economic benefits the United Nations designated the year 2004 as the International Year of Rice [1]. The high- yielding varieties of rice introduced from the plain or hill regions of the northern and southern parts of the country do not perform well in north eastern hill region [2]. The local varieties provide security against crop failure and wide cropping windows. They show good tolerance to local environmental conditions. The importance of local land races of rice in breeding programme lies in the evolution of gene complexes in the local cultivars that have co adapted to specific local environment through long period of natural selection. It is not easy to study the function of individual genes and concerted efforts are needed to analyze the interaction among themselves as well as with the environment. The modern recombinant DNA technology may not be very helpful in reconstitution if these gene complexes [3, 4]. Mutation may produce raw material which can be used for the genetic improvement of crops [5]. Ethyl methanesulfonate (EMS) is a mutagenic teratogenic organic compound. EMS is one of the most frequently used alkylating agent for chemical mutagenesis in plants due to its potency and ease with which it can be used. EMS is more effective than physical mutagens [6, 7]. It is mainly used to produce GC to AT transition [8, 9] EMS results in stable point mutations and thus produces an allelic series of missense changes that can provide a range of phenotypes. The present investigation was done to determine the effects of different doses of ethyl methanesulfonate on seed germination in two varieties of rice *viz*. Ahu and Baismuthi.

## Materials and Methods
The experimental material consists of two indigenous rice varieties of Assam - Ahu and Baismuthi. Ethyl methanesulfonate is used as mutagenic agent. 50 ml of 0.5%, 1%, 1.5%, 2% concentrations of Ethyl methanesulfonate (w/v) were prepared in water. The pH of the solutions was adjusted at 7.
Seeds of Ahu and Baismuthi variety of Rice were each placed in 16 petri dishes (8 petri dishes each) and double distilled water was added to a level above the seeds. Seeds were soaked overnight at room temperature for 20 hours. Subsequently, the water was decanted and 50 ml of 0.5%, 1%, 1.5%, 2% and a control dose of 0% concentrations of EMS (v/v) in water was added. Seeds were kept for 5 hours at room temperature followed by decantation of the EMS and rinsing with 100 ml of double distilled water (5 times, 4 minutes each). Seeds were then rinsed under running tap water for 4 hours before planting in Petri dishes. Following the next day of the treatments, the seeds were continuously assessed for the germination and developmental stages daily. The seedlings were then transferred in plastic pots and grown in the botanical garden under natural conditions.
The following parameters were studied for seedling growth under laboratory conditions

### Germination Percentage
The seeds sown in the petridishes were grown under room temperature and observed daily until it reaches maximum generation. After seven days, the number of seeds

germinated under this condition was recorded and was followed on 14th day from the day of sowing in laboratory. The germination percentage was calculated by using the following equation:

$$\text{Germination (in percent)} = \frac{\text{No. of seedling emerged}}{\text{Total no. of seed sown}} \text{ X } 100$$

**Shoot and Root Length (In Cm)**

The growth of seedlings in terms of its height was also measured after 16 days from the day of sowing under laboratory conditions.

**Root Length (In Cm)**

The root length was measured on the 16th day from the date of sowing.

**Number of Roots**

The number of roots was counted on the 16th day from the date of sowing.

**The Following Parameters Were Studied for Seedling under Field Conditions**

The seedlings grown in the petridishes under room temperature were transferred to plastic pots on 17th day from the day to sowing. Seedlings from each EMS concentration applied and those of the control were transferred and planted in the rice field soil prepared in plastic pots. Also the plants were watered regularly with normal tap water. The seedling heights, number of tillers, number of root and the root length of the plants were measured from the day of transplantation.

**Seedling Height (In Cm)**

The effects of ethyl methanesulfonate on seedlings height of each individual from every treatment of both the varieties was measured on 12th day from the day to transplantation under field conditions.

**Number of Tillers**

The alkylating agent EMS effects on number of tillers were observed under field conditions. The observation was done on the 12th day from the day of transplantation. Mean values of each treatment and control were calculated from the recorded data from each individual under the respective treatments. The mean values of different treatment were compared with that of the control to see the effects of mutagen.

**Number of Roots and Root Length**

For observing root growth in terms of its number and length, the plants were uprooted slowly. It was done by loosening the soil under water with hand and pulling the plant up slowly one at a time. Care should be taken to avoid breakage of the roots observation were carried out once as in the case of all parameters.

**Statistical Analysis**

Statistical analysis was performed using one-way ANOVA (for $P<0.05$).

## Results and Discussion

**Germination Percentage**

Seed germination in both the varieties decrease due to the effects of EMS with considerably increase in the higher levels of doses. This reduction was found to be dose dependent in both the varieties. Similar observations were observed by many workers in *Vigna radiate* [10, 11] Inhibition in seed germination after mutagenic treatment has been attributed to changes in biochemical and physiological system [12] and inhibitory effect of mutagen [13]. The reduction percentage in seedling germination ranges from 12.5% -67.5% in Ahu and 16.25%-71.25% in Baismuthi Thus, the stimulating effect of physical mutation on germination may be credited to the activation of RNA or protein synthesis. It may occur during the early stage of germination after the seeds are treated as also observed by Abdel *et al.* [14]

**Table 1:** Effect of Different Treatment of Ems on Seed Germination of Both the Varieties

| Germination Percentage | | | | |
|---|---|---|---|---|
| Mutagen conc. (%) | Observation on 7th Day | | Observation on 14th Day | |
| | Ahu | Baismuthi | Ahu | Baismuthi |
| Control | 87.5 | 85 | 100 | 100 |
| 0.5 | 62.5 | 63.75 | 87.5 | 83.75 |
| 1 | 37.5 | 40 | 55 | 52.5 |
| 1.5 | 25 | 26.25 | 42.5 | 35 |
| 2 | 17.5 | 18.75 | 32.5 | 28.75 |

**Seedling Height**

**Table 2:** Effect of Different Treatments of Ems on Seedling Height (In Cm) of Both the Varieties under Laboratory and Field Conditions.

| | Under laboratory conditions | | Under field conditions | |
|---|---|---|---|---|
| Mutagen conc. (%) | Ahu | Baismuthi | Ahu | Baismuthi |
| Control | 4.18 | 5.26 | 42.58 | 38.98 |
| 0.5% EMS | 5.29 | 4.9 | 46.54 | 42.96 |
| 1% EMS | 5.02 | 4.61 | 38.02 | 31.56 |
| 1.5% EMS | 3.76 | 4.48 | 49.32 | 27.88 |
| 2% EMS | 3.66 | 5.82 | 49 | 41.4 |

The table 2 shows that there is a decrease in seedling height with the increase in EMS concentration. The decrease is more significant in Baismuthi variety under field condition. But at 2% EMS the seedling height in both the varieties increased significantly. Under laboratory conditions there is a significant decrease in seedling height of Baismuthi at 0.5%, 1% and 1.5%. But there is an increase in Ahu variety at 0.5% and 1%. These findings are in close agreement with that of the earlier reports on chemical mutagen, effects of sodium azide by Wang and Yu [15], Solanki and Sharma [16] and Kumar and Selvaraj [17]. The enhancing effect may be due to sudden increase in the metabolic status of seedlings and increase in the activity of growth promoters. Reduction in biological criteria plant height may be attributed to a drop in auxin level, inhibition of auxin synthesis [18].

**Root Length**

The evaluation of root length under laboratory conditions was done on 16th day from the day of showing and under

field conditions was done on 12th day from the day to transplantation under field conditions. A gradual reduction in root length was observed in both the varieties in all the treatments except at 2.0% EMS in the variety Baismuthi. Under field conditions the root length in both the varieties in all the treatments, except at 1.5% EMS in Baismuthi, is reduced significantly. A significant effect of the concentration of applied EMS on the root length in variety Ahu was observed under laboratory conditions. Reduction in root length occurred with each corresponding increase in the concentration of EMS. The effects observed under the low-or high-dosage treated plants are enhancement or inhibition of seedling/plant growth, root length and other biological responses. This is in contrast with earlier findings [19] that, an increase in chemical concentration, there was an increase in the rate of mutation leading to variations in rooting whereas under field conditions, the two varieties showed a more or less same response to root length reduction. Similar results have also been reported for *Capsicum annuum* [20] and *Avena sativa* [21] when treated with EMS and dimethyl sulfate; EMS and azide, respectively.

**Table 3:** Effect of different treatments of EMS on root length (in cm) of both the varieties under laboratory and field conditions.

| | Under laboratory conditions | | Under field conditions | |
|---|---|---|---|---|
| **Mutagen conc (%)** | **Ahu** | **Baismuthi** | **Ahu** | **Baismuthi** |
| Control | 4.04 | 5.72 | 24.48 | 23.38 |
| 0.5% EMS | 3.74 | 4.95 | 17.14 | 23 |
| 1% EMS | 3.30 | 4.12 | 16.36 | 20.24 |
| 1.5% EMS | 2.37 | 4.86 | 22.16 | 24.2 |
| 2% EMS | 1.60 | 6.02 | 20.46 | 19.7 |

**Number of Roots**

**Table 4:** Effect of different treatments of EMS on number of rootlets under laboratory and field conditions

| | Under laboratory conditions | | Under field condition | |
|---|---|---|---|---|
| **Mutagen** | **Ahu** | **Baismuthi** | **Ahu** | **Baismuthi** |
| Control | 3 | 3.2 | 67.6 | 61.8 |
| 0.5% EMS | 3.6 | 4.93 | 75.8 | 75 |
| 1% EMS | 4.13 | 3.8 | 34.6 | 54.6 |
| 1.5% EMS | 2.53 | 1.93 | 67.2 | 36.4 |
| 2% EMS | 2.53 | 3.86 | 83.4 | 59 |

Number of rootlets and roots increases significantly under lower doses of EMS in both varieties under laboratory conditions and gradually reduces its number with increase in EMS doses (i.e. from 1.5%- 2%) except for Baismuthi which increases again at 2% EMS. Thus this effect of mutagen on rootlets can led to the indication of effective mutagenesis that can result in formation of desirable traits. Under field condition, the root length in both the varieties in all the treatments, except at 1.5% EMS in Baismuthi, is reduced significantly. The highest reduction recorded in Ahu is 29.98% at 0.5% EMS while it was 20.46% at 2.0% EMS in Baismuthi.

**Number of Tillers Per Plant**

On the 29th day after transplantation, there is an overall reduction in comparison to controls in number of tillers in both the varieties. Reduced growth due to higher doses was also explained differently by different workers. It may be attributed to one or more of the following reasons:(i) the increase in growth promoters, (ii) the sudden increase in metabolic status of seeds at certain levels of dose, (iii) the increase in destruction of growth inhibitors, (iv) drop in the auxin level or inhibition of auxin synthesis and (v) decline of assimilation mechanism. The variation in biological parameter *viz*. no of tillers may be attributed to a drop in auxin level [22] or due to decline of assimilation mechanism [23].

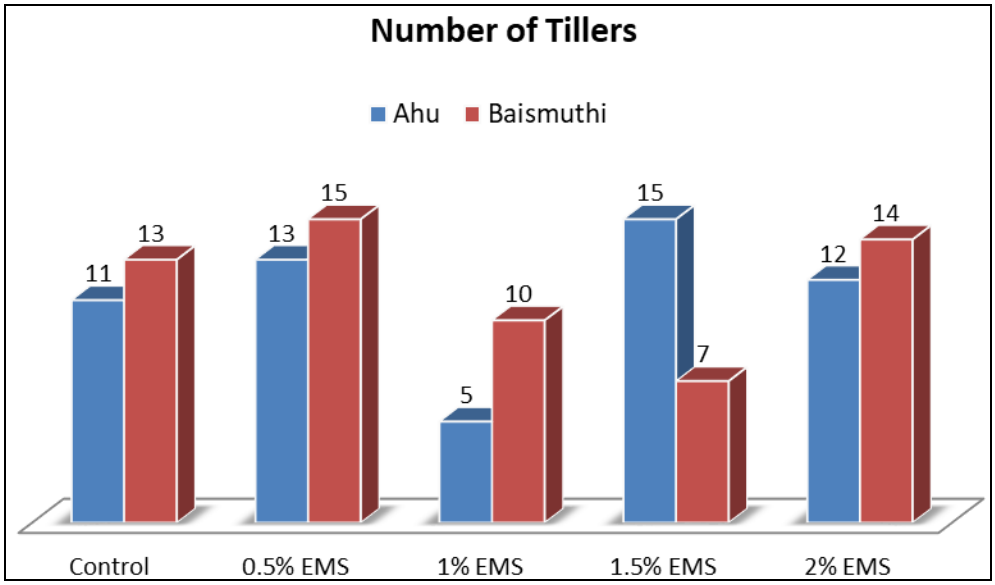


**Fig 1:** Maximum number of tillers observed in each concentration as on 29th day after plantation.

**Conclusion**

Morphological traits such as plant length and root numbers were decreased with concentration of EMS and treatment duration increase. Similar results were reported by Ramachandra *et al.*[24], who showed that there was a decrease in the growth parameters with increase in the concentration of EMS solution, which may be due to the fact that, the enzymatic activity associated with the biosynthesis of primary metabolic traits might have been reduced with increased concentration of EMS solution.

Various investigations have documented a negative relationship between seed germination and concentrations of EMS. Therefore, it is recommended to perform a dose-response analysis to determine the average lethal dose of EMS. Thus with the above observations and findings, we can compare the effects of mutagen EMS on the two rice varieties where variations were observed between them and under which concentration it acts more in enhancing various traits and this can also be interpreted with other different varieties of crops for generating new improved varieties.

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