



Combined Effect of Multiple Chemical Mutagens and Treatment Duration on the Germination of Rice Hybrids in M_1F_1 Generation

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ABSTRACT

Background: Mutation breeding is one of the important techniques for creating genetic variability and developing improved crop varieties with desirable traits. Chemical mutagens such as ethyl methanesulfonate (EMS), sodium azide (NaN₃), colchicine and organic solvent dimethyl sulfoxide (DMSO) have been frequently used in plant mutation breeding programs. However, the combined effects of multiple chemical mutagens and their treatment durations on plant germination, growth and development are not well understood.

Methods: This study investigated the combined effects of chemical mutagens comprising of EMS, Sodium azide, colchicine and DMSO on the germination of five commercial rice (*Oryza sativa* L.) hybrids in the M₁ generation. Seeds were treated in the mutagen solution for varying durations ranging from 4 to 24 hours.

Result: Significant differences were observed in germination rates among the hybrids and across treatment durations. The highest germination rate of 3.08% was observed in Arize 6444 Gold with a 4-hour treatment, while the lowest rate of 0.17% was recorded in Srichakra 1168 with a 24-hour treatment. Overall, germination rates decreased with increasing treatment duration, likely due to the cumulative mutagenic effects and DNA damage. Srichakra 1168 exhibited the highest tolerance to the mutagen mix, while DPS-Virat was the most sensitive. Despite the low germination rates, the surviving M₁ plants may harbor a range of desirable and undesirable mutations, presenting opportunities for identifying valuable mutants with improved traits in subsequent generations. This study highlights the importance of optimizing mutagen combinations and treatment conditions to balance the induction of genetic variability with the maintenance of seed viability in mutation breeding programs for rice improvement.

Key words: Chemical mutagens, Germination, Hybrids, Mutation, Rice.

INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most important cereal crops worldwide, serving as a staple food source for more than 3 billion of the global population (Hashimoto *et al.*, 2022). Improving rice yields and developing new rice varieties with desirable traits such as increased resistance to biotic and abiotic stresses, better grain quality and higher nutritional value are crucial for meeting the growing food demands of an ever-increasing population. Mutation breeding is a valuable tool in plant breeding programs aimed at generating genetic variability and developing improved crop varieties (Dorvlo *et al.*, 2022). Chemical mutagens, such as ethyl methanesulfonate (EMS), colchicine and sodium azide, have been widely employed to induce random mutations in plant genomes, leading to the generation of novel genetic variants with potentially beneficial traits (Oladosu *et al.*, 2016). The rationale behind using multiple chemical mutagens lies in their different modes of action and the types of mutations they induce. By combining mutagens with diverse mechanisms, such as alkylating agents, base analogs and chromosome-breaking agents, a broader spectrum of genetic changes can be achieved (Chaudhary and Kumar, 2023; Mishra *et al.*, 2024). EMS is an alkylating agent widely used as a chemical mutagen in plant breeding programs. It primarily induces point

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mutations by alkylating guanine bases, leading to their mispairing with thymine during DNA replication. This results

in the formation of random single-base pair substitutions throughout the genome (Greene *et al.*, 2003; Novak, 1992). Sodium azide is a powerful mutagen that primarily causes chromosomal aberrations, including deletions, inversions and translocations. It is known to induce DNA double-strand breaks and interfere with the cellular repair mechanisms, leading to the formation of complex chromosomal rearrangements (Mir and Jan, 2023). Colchicine is a mitotic spindle inhibitor that prevents the proper separation of chromosomes during cell division. This can lead to the formation of polyploid cells, where the chromosome number is doubled or multiplied (Blakeslee and Avery, 1937; Surson *et al.*, 2024). DMSO is an organic solvent commonly used as a carrier or penetrating agent in chemical mutagenesis experiments. While DMSO itself is not a potent mutagen, it can enhance the mutagenic effects of other chemical mutagens by facilitating their penetration into cells and tissues (Amin *et al.*, 2015). DMSO is often used in combination with other mutagens, such as EMS or sodium azide, to improve their uptake and mutagenic efficiency in plant mutagenesis studies. It is important to note that the mutagenic effects of these chemical agents can vary depending on the plant species, treatment conditions e.g., concentration, duration and stage of application and the specific combinations used (Jerish *et al.*, 2025; Oladosu *et al.*, 2016). The simultaneous application of these mutagens can lead to a higher frequency of both point mutations and chromosomal rearrangements, potentially increasing the chances of obtaining desirable mutant phenotypes (Bado *et al.*, 2015).

Hybrids possess a higher level of genetic diversity and heterozygosity because they are developed from different parent lines having different combination of genes. This increased heterozygosity can provide a buffering effect against deleterious mutations induced by mutagenic treatments, reducing the likelihood of severe phenotypic consequences (Rasmusson and Phillips, 1997). Mutagenic treatments can induce a wide range of genetic variations in the hybrid population. As these populations segregate in subsequent generations, the genetic variability increases, providing a larger pool of potential mutants with diverse traits (Kharkwal and Shu, 2009).

In traditional plant breeding program, the combined effects of multiple chemical mutagens on growth and development of hybrid are not well understood. It is

possible that the simultaneous application of different mutagens could lead to synergistic or antagonistic effects, influencing the efficacy of the mutagenesis process and the resulting phenotypic variations. In this study, we investigated the combined effects of various chemical mutagens including EMS, Colchicine and Sodium azide, along with DMSO for multiple treatment hours on the germination of rice hybrids.

MATERIALS AND METHODS

The research was conducted during the Kharif season of 2023 at the Rice Research Farm, BAU, Kanke Ranchi. For each of the five commercial rice hybrids, 1 kg of seeds was divided into six equal portions of 166.67 grams and stored in labeled transparent polybags. A mutagen solution was prepared by combining Colchicine (1 g), Ethyl Methanesulfonate (10 g) and Sodium azide (25 g) in 2.5 liters of distilled water containing 0.1% DMSO solution. The seed packets from each hybrid variety were subjected to different treatment durations of 4, 8, 12, 16, 20 and 24 hours in the prepared mutagen solution. Following each specified treatment duration, the seeds were thoroughly rinsed with distilled water to remove any residual chemicals. Subsequently, the treated seeds were sown in the field to establish the mutant population and data on seedling germination was collected from these M₁F₁ populations.

Germination per cent =

$$\frac{\text{Number of seeds germinated}}{\text{Total number of seeds sown}} \times 100$$

RESULTS AND DISCUSSION

The germination of different rice hybrids varied significantly after treatment with the multiple chemical mutagen mix comprising of ethyl methanesulfonate, sodium azide and colchicine along with DMSO for different treatment durations in the M₁ generation. The data on plant germination rates are presented in Table 1. The approximate number of seeds per treatment hour is presented in Table 2 for each hybrid which is calculated based on the test weight.

The highest plant germination rate of 3.08% was observed in the Arize 6444 Gold when treated with the mutagen mix for 4 hours, while the lowest germination rate of 0.17% was recorded in the Srichakra 1168 in 24-hour treatment. In general, the germination rate decreased with

Table 1: Number of plants germinated in each hybrid for different treatment duration.

Treatment hours	Number of plants germinated (out of 1 Kg of seed sown)				
	DPS-virat	Delta speed	Arize 6444 gold	Srichakra 1168	PAC 807+
4	30	40	160	96	98
8	21	22	80	73	73
12	18	21	40	71	49
16	18	22	14	60	24
20	14	17	16	26	16
24	11	12	14	9	12
Total	112	134	324	335	262

an increase in the treatment duration for all hybrid combinations (Fig1). Among the hybrids, Srichakra 1168 exhibited the highest tolerance to the combined effect of mutagens as with 6.44% germination rate, with percentage of plants survived ranging from 0.17% (24 hours) to 1.85% (4 hours). On the other hand, DPS-Virat was the most sensitive, with germination percentage ranging from 0.21% (24 hours) to 0.57% (4 hours).

The results clearly demonstrate the differential response of rice hybrids to the combined effect of chemical mutagens and the impact of treatment duration on plant germination in the M₁ generation. The observed variation in germination rates can be attributed to the inherent genetic makeup of the hybrids and their respective tolerance levels to the mutagenic agents.

The decrease in germination rate with increasing treatment duration can be explained by the cumulative mutagenic effects on the rice genome, leading to higher levels of DNA damage and cellular stress (Akilan *et al.*, 2020; Mohammed *et al.*, 2018). Emrani *et al.* 2011 also reported decrease in germination percentage with increasing mutagen doses and treatment hour in canola. Reduced germination percentage with increase in EMS concentration also reported in rice (Shamshad *et al.*, 2023), sesame (Parthasarathi *et al.*, 2020), okra (Baghery *et al.*, 2016), pigeon pea (Ariman *et al.*, 2014), tomato (Sikder *et al.*, 2013), sunflower (Habib *et al.*, 2021), soyabean

(Nilahayati *et al.*, 2023), mungbean (Ali *et al.*, 2024). Prolonged exposure to the chemical mutagens may have induced higher frequencies of deleterious mutations, chromosomal aberrations and physiological impairments, ultimately resulting in reduced plant germination (Chaudhary and Kumar, 2023; Mir and Jan, 2023; Mishra *et al.*, 2024).

The Srichakra 1168 demonstrated remarkable resilience to the combined mutagenic treatment, suggesting the presence of sophisticated genetic mechanisms or DNA repair pathways that provide enhanced protection against the deleterious effects of chemical mutagens. This superior tolerance could be attributed to various factors, including the genetic architecture inherited from its parental lines, the presence of specific stress-response genes, or the synergistic interaction of multiple protective mechanisms within the hybrid genome. The robust performance of Srichakra 1168 under mutagenic stress makes it an excellent candidate for further investigation in mutation breeding programs, as it could potentially generate a diverse array of beneficial mutations while maintaining acceptable levels of seed viability and germination rates. This characteristic is particularly valuable in mutation breeding, where the balance between inducing genetic variation and maintaining plant survival is crucial for success.

Conversely, DPS-Virat exhibited pronounced sensitivity to the mutagenic treatment, displaying significantly reduced germination rates and increased susceptibility to the chemical mutagens. This heightened sensitivity could be traced back to several factors, including the specific genetic composition inherited from its parental lines, potentially compromised DNA repair mechanisms, or the absence of crucial stress-tolerance genes. The genetic background of DPS-Virat might lack robust cellular defense systems or possess certain genetic elements that make it more vulnerable to DNA damage and chromosomal aberrations induced by the chemical mutagens. Understanding the

Table 2: Test weight and approximate number of seeds of each hybrid.

Hybrids	Test weight (1000 seeds weight)	Estimated no of seeds sown in 166.67 g (Approx.) for each treatment hour
DPS-virat	30	5300
Delta speed	31	4900
Arize 6444 gold	32	5200
Srichakra 1168	29	5200
PAC 807+	30	5900

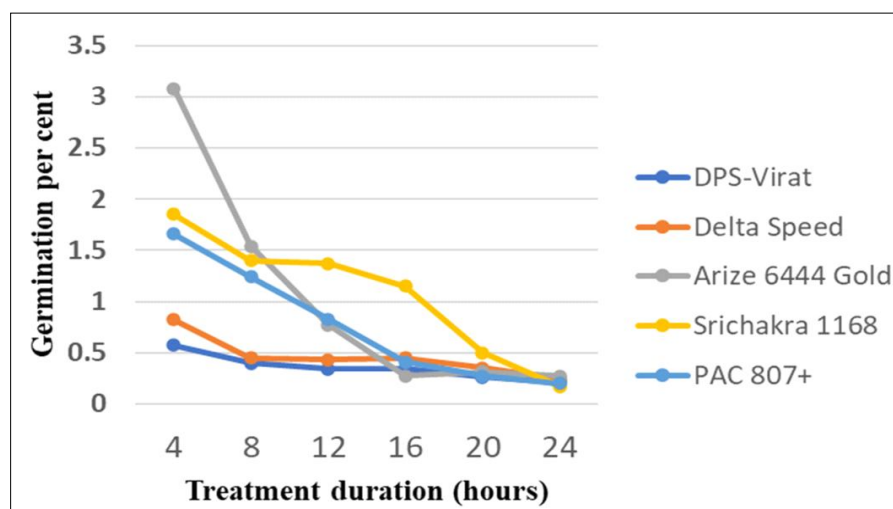


Fig 1: Decreasing trend of germination per cent of rice hybrid with mutagens treatment duration.

molecular basis of this sensitivity could provide valuable insights into the mechanisms of mutagenic action and plant stress responses, potentially leading to improved strategies for mutation breeding in sensitive genotypes.

The striking variations observed among the different hybrids and their responses to various treatment durations emphasize the critical importance of careful genotype selection and precise optimization of mutagenic treatments in mutation breeding programs. These differences underscore the complex interplay between genetic background and mutagenic sensitivity, suggesting that successful mutation breeding requires a nuanced approach tailored to specific genetic materials. The optimization process should consider multiple factors, including the genetic constitution of the breeding material, the desired mutation frequency, the specific traits targeted for improvement and the overall breeding objectives. By carefully calibrating treatment conditions based on hybrid-specific responses and incorporating knowledge about genetic repair mechanisms and stress tolerance, breeders can develop more efficient mutagenesis protocols that maximize the generation of useful genetic variation while minimizing the negative impacts on seed viability and plant development. This tailored approach would significantly enhance the effectiveness of mutation breeding programs, leading to more successful development of improved crop varieties with desirable agronomic traits.

CONCLUSION

Combined effect of multiple chemical mutagens along with DMSO have reduced the germination of rice hybrids with prolonged treatment duration but, the surviving M₁ plants may harbor a range of mutations, both desirable and undesirable, which will be expressed and segregated in subsequent generations. Despite very low germination rate, there is high chances of generation of mutants with desirable features. By carefully screening and evaluating these mutants in subsequent generations, it is possible to identify and select mutants exhibiting desirable characteristics, such as improved yield, resistance to biotic and abiotic stresses, or enhanced nutritional quality. The library of mutants with desirable morpho-agronomic traits may be created that can be beneficial in crop improvement programs in future and attaining sustainable agriculture at a higher pace. Future studies could involve molecular characterization of the induced mutations, assessment of agronomic and quality traits in advanced generations and the development of strategies for efficient mutation breeding in rice and other crop species.

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Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent

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Conflict of interest

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