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The Effect of Chemical Mutagens (*Ethyl Methane Sulfonate* and Oryzalin) on Germination of Ratna Flowers (*Gomphrena Globosa* L.)

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ABSTRACT: The results showed that EMS and Oryzalin treatments affected all observed parameters. These two chemical mutagens had an impact on plumule length, radicle length, sprout color changes, germination capacity and percentage of abnormal sprouts. EMS treatment resulted in plumule lengths of 2.8 cm and 2.6 cm in purple and pink genotype, respectively while Oryzalin produced only 1.3 cm for both genotypes. Similarly, for radicle length, EMS resulted in 1.2 cm and 1.3 cm, while Oryzalin yielded only 0.9 cm for both genotypes. This indicates that EMS tended to retain some of the primary growth capacity, whereas Oryzalin significantly inhibited the growth of both the plumule and radicle. In terms of germination capacity, EMS maintained relatively high viability, 85% in the purple genotype and 75% in the pink genotype, while Oryzalin reduced it to 70% in both genotypes. However, the rate of abnormal germination was higher in the Oryzalin treatment at 75% compared to EMS at 60-65%. These findings indicate that EMS and Oryzalin mutagens are capable to induce random mutations that affect early growth processes, starting from the germination stage.

KEYWORDS: EMS, Oryzalin, Germination, *Gomphrena globosa* L.

INTRODUCTION

Ratna flower (*Gomphrena globosa* L.) is a native plant from America and is now widely cultivated in Indonesia as an ornamental plant [1]. It belongs to the Amaranthaceae family that can grow throughout the year and sometimes appearing as a wild plant in areas that receive adequate sunlight. In Bali, this flower is commonly used for decorative purposes in entertainment, sacred events and religious ceremonies [2]. Ratna flowers have plenty of potential for development into various products, including food, medicine, ornamental plants and cut flowers (floriculture).

One of the plant breeding methods used to produce unique ornamental varieties and enhance plant diversity is induced mutation. *Ethyl Methane Sulfonate* (EMS) and Oryzalin are chemical mutagens widely used in biotechnology and plant breeding. Both agents contribute to increased genetic variation and improved plant adaptation. EMS is a chemical mutagen that induces point mutations by altering guanine bases, causing them to pair incorrectly during DNA replication. These effects are typically random and can lead to specific genetic changes in certain genes without directly damaging the chromosomes [3]. Meanwhile, Oryzalin is a mutagen that disrupts microtubules formation during cell division, interfering with normal chromosome segregation. This process leads to polyploidization, or an increase in the chromosomes number in a cell, which can affect overall plant size and growth [4]. The choice of mutagen depends on the purpose of the research or application, as well as the tolerance for genetic modifications.

Soaking flower seeds using chemical mutagens can influence both morphological and physiological traits. The impact of these mutagens on germination can be observed through variations in plumule and radicle length, germination rate, percentage of abnormal sprouts, and cotyledon color.

RESEARCH METHODS

This research was conducted in Bali from June to July 2024 at the Experimental Garden of the Faculty of Agriculture, Udayana University. The study used a two-factor Completely Randomized Design (RAL), consisting of Genotype and Chemical Mutagen factors, with four treatment combination, namely GUM1 (Purple Genotype + EMS), GUM2 (Purple Genotype + Oryzalin), GPM1

(Pink Genotype + EMS), and GPM2 (Pink Genotype + Oryzalin). Each treatment was replicated six times. Data were analyzed using Analysis of Variance (ANOVA), and followed by the Least Significant Difference (LSD) test at the 5% significance level.

The experimental procedure comprised several stages, including material preparation, mutagen solution preparation, seed soaking, seed incubation in moist tissue media, sprout observation, and data collection. The control treatment was conducted separately prior to the chemical mutagen applications. The EMS 1% solution was prepared by adding 0.2 mL of pure EMS into a measuring tube and diluting it with distilled water to reach a final volume of 20 mL. The Oryzalin 1% solution was prepared by dissolving 0.2 g of Oryzalin powder in 1 mL absolute ethanol, then diluted with distilled water to a final volume of 20 mL. Germination tests were conducted using the UKDdP method (Rolled Paper Test established in Plastic) [5], with the modification in which the paper medium was replaced by bamboo tissue. The tissue was evenly moistened using a sprayer to achieve optimal humidity. Ratna flower seeds that have been soaked with EMS and Oryzalin were arranged in a row on the surface of the tissue with sufficient distance and neat so as not to overlap each other. Next, the paper towel is gently rolled by ensuring that the position of the seeds does not shift. Each roll was wrapped in plastic wrap to create a moist and dark environment that favors germination. The rolls were stored in a shaded location that is not exposed to direct light. Throughout the observation period, the tissue was re-moistened as needed to maintain consistent humidity and prevent the sprouts from drying out until the end of the research process. After the incubation period, observations were made on all tested seeds. The parameters measured included plumule length (cm), radicle length (cm), germination capacity (%), percentage of abnormal sprouts (%), and cotyledon color changes.

Germination capacity refers to the percentage of Ratna flower seeds that successfully germinate under normal conditions within a certain period of time. Germination capacity is calculated based on the percentage of seeds that grow normally to the total number of seeds.

$$\text{Germination Capacity (\%)} = \left(\frac{\text{number of normally grow seeds}}{\text{total number of seeds}}\right) \times 100$$

Percentage of abnormal sprouts was determined to assess the proportion of seeds that germinated but exhibited morphological abnormalities, such as twisted plumules, undeveloped radicles, or asymmetrical shapes. Abnormal germination is calculated based on the number of seeds that grow with abnormal morphology compared to the total number of seeds.

$$\text{Abnormal Sprouts (\%)} = \left(\frac{\text{number of abnormally grow seeds}}{\text{total number of seeds}}\right) \times 100$$

RESULTS

Effect of Chemical Mutagens on the Length of Plumule and Radicle of Ratna Flower

Observations conducted on the 10th day after sowing revealed significant differences in plumule and radicle length among the treatments, indicating that the two mutagens had distinct effects on early growth process. EMS treatment resulted in a plumule length of 2.8 cm in the purple genotype and 2.6 cm in the pink genotype. Corresponding radicle length was 1.2 cm and 1.3 cm, respectively. Meanwhile, Oryzalin treatment yielded lower plumule lengths of 1.3 cm (purple) and 1.2 cm (pink), and radicle length of 0.9 cm in both genotypes. These findings suggest that EMS had a more favorable effect on the initial development of both the plumule and radicle compared to Oryzalin, which appeared to significantly inhibit early seedling growth (Table 1).

Table 1. The Effect of Chemical Mutagens on the Length of Plumule (cm) and Radicle (cm) of Ratna Flowers (*Gomphrena globosa* L.)

Treatments	Plumule Length (cm)	Radicle Length (cm)
GUM0	3,4 a	2,6 b
GUM1	2,8 ab	1,2 a
GUM2	1,3 b	0,9 a
GPM0	3,3 a	2,5 b
GPM1	2,6 ab	1,3 a
GPM2	1,3 b	0,9 a
BNT 5%	0,42	0,14

Note: The means followed by the same letter at the same column are not significantly different according to LSD (Least Significant Different) test at alpha 5%.

The Effect of Chemical Mutagens (Ethyl Methane Sulfonate and Oryzalin) on Germination of Ratna Flowers (Gomphrena Globosa L.)

Effect of Chemical Mutagens on Germination Capacity and Percentage of Abnormal Sprouts of Ratna Flower

Soaking Ratna flower seeds in EMS and Oryzalin solutions affected both germination capacity and abnormal sprouts. EMS treatment resulted in a reduction in germination capacity, with values of 85% in the purple genotype and 75% in the pink genotype. Additionally, the percentage of abnormal sprouts increased to 60% in the purple genotype and 65% in the pink genotype.

Oryzalin treatment also reduced germination capacity to 70% in both genotypes, accompanied by an increase in abnormal sprouts of 75% in the purple genotype and 70% in the pink genotype. These findings indicate that both chemical mutagens have a negative impact on seed viability and early seedling development, as reflected by reduced germination and increased morphological abnormalities (Table 2).

Table 2. The Effect of Chemical Mutagens on Percentage of Germination Capacity (%) and Abnormal Sprout (%) of Ratna Flowers (*Gomphrena globosa* L.)

Treatments	Germination Capacity (%)	Abnormal Sprout (%)
GUM0	90	20
GUM1	85	60
GUM2	70	75
GPM0	85	25
GPM1	75	65
GPM2	70	70

Note: Simple calculation results according to the method.

Effect of Chemical Mutagens on Sprouts Color of Ratna Flower

Qualitative observation conducted on the 10th day after germination revealed that the Sibanggede genotype exhibited purple-colored cotyledons. The control treatment shows that both genotypes have purple cotyledon color. After EMS treatment, changes in cotyledon color were observed: the purple genotype exhibited a lighter purple hue, while the pink genotype showed a light yellowish-purple color. Oryzalin treatment also induced cotyledon color changes in both genotypes, resulting in a darker shade of purple. These changes suggest that both chemical mutagens influenced pigmentation expression during early seedling development (Table 3).

Table 3. The Effect of Chemical Mutagens on Sprouts Color of Ratna Flower (*Gomphrena globosa* L.)

Treatment	Replication					
	I	II	III	IV	V	VI
GUM0	Purple	Purple	Purple	Purple	Purple	Purple
GUM1	Light Purple	Light Purple	Light Purple	Light Purple	Light Purple	Light Purple
GUM2	Dark Purple	Dark Purple	Dark Purple	Dark Purple	Dark Purple	Dark Purple
GPM0	Purple	Purple	Purple	Purple	Purple	Purple
GPM1	Light Purple-Yellow	Light Purple-Yellow	Light Purple-Yellow	Light Purple-Yellow	Light Purple-Yellow	Light Purple-Yellow
GPM2	Dark Purple	Dark Purple	Dark Purple	Dark Purple	Dark Purple	Dark Purple

Note: Color observations are adjusted visually and with the RHS Color Chart.

Effect of Chemical Mutagens on Germination and Early Growth of Ratna Flower

Morphological observations of the Ratna flower sprout 10th day after sowing revealed distinct differences between genotypes and mutagen treatments. In the control sprouts (a and b), both purple and pink genotypes produced sprouts with normally developed plumules and radicles that grew straight, long, and with a distinctive and bright cotyledon color. EMS treatment (c and d) exhibited paler cotyledon colors and thinner elongated plumules. Mild deformation was observed particularly in the pink genotype, suggesting differential sensitivity to EMS. Oryzalin treatment (e and f) showed cotyledons with higher color intensity and sprouts with shorter and distorted shape. These morphological changes in color and shape reflect the specific mutagenic responses induced by each chemical agent and highlight the varying degrees of genotype tolerance to mutagenic stress.

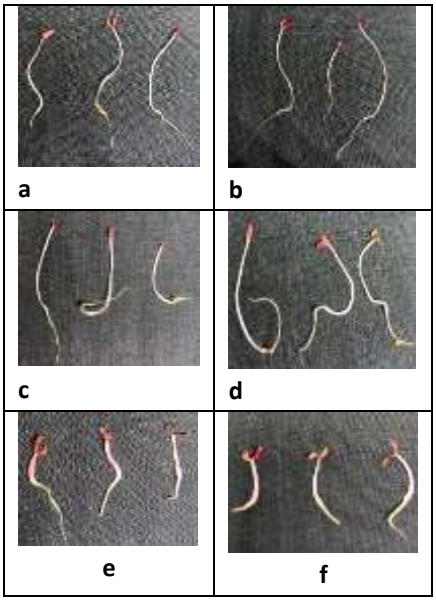

Figure 1. Ratna Flower 10th Day After Sowing ((a) purple control, (b) pink control, (c) EMS purple, (d) EMS pink, (e) Oryzalin purple, (f) Oryzalin pink)

Differences in the growth of Ratna flower seedlings due to mutagen treatment on five weeks after transplanting showed that control plants (a and b) grew normally with bright green leaves and genotypically colored stems (purple or pink). EMS treatment (c and d) displayed morphological abnormalities, including smaller, curled, and cup-shaped leaves, with paler colored stems than the control. Oryzalin treatments (e and f) produced seedlings with broader and thicker leaves and along with intensified coloration, which are indicative of polyploidization effects. However, the growth rate of the seedlings tended to be slower than the control, indicating that genome duplication induced by Oryzalin may increase organ size without accelerating development.

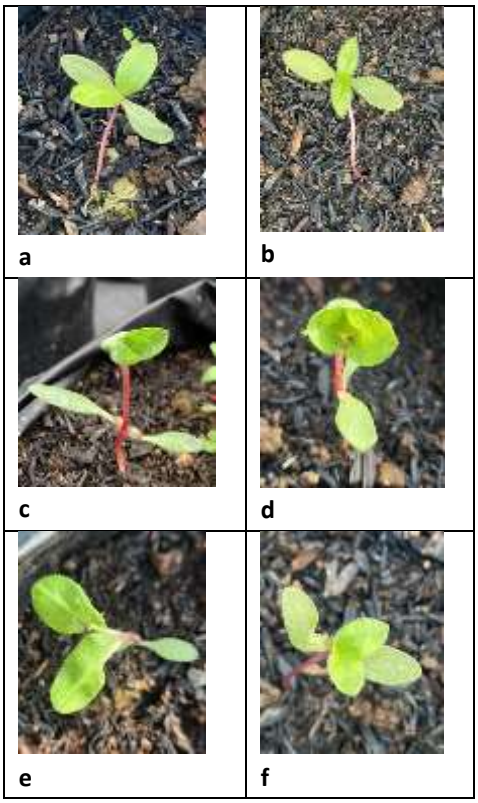

Figure 2. Ratna Flower Seedlings 5 Weeks After Planting ((a) purple control, (b) pink control, (c) purple EMS, (d) pink EMS, (e) purple Oryzalin, (f) pink Oryzalin)

DISCUSSION

Immersion of Ratna flower seeds in EMS and Oryzalin solution significantly affected plumule and radicle length. EMS 1% treatment of Ratna flowers positively influenced root system development during the early growth phase, characterized by longer plumules and radicles due to the induction of point mutations that regulate of meristematic tissue growth. The impact of the mutation leads to morphological deformation, such as root deformation or abnormal growth of sprouts and reduced germination capacity [6]. At higher concentrations, EMS tends to suppress plumule and radicle elongation due to its cytotoxicity, which interferes with cell division and elongation processes. EMS can inhibit plumule elongation due to DNA damage in apical meristem cells, which play a crucial role in shoot development. In radicle growth deformation occurs due to DNA damage or apoptosis process in root meristem cells. This toxic effect is more pronounced in the radicle compared to the plumule, as roots are the first organs to exhibit active growth during the germination process [7]. Despite its toxicity, the results showed that the germination capacity of EMS treatment was still higher than those treated with Oryzalin, indicating that EMS has lesser impact on seed viability.

Meanwhile, Oryzalin 1% produced shorter, thicker and more uniform plumules and radicles compared to EMS, indicating that Oryzalin had the mechanism of action in inducing polyploidy begins to manifest during the germination phase. These findings suggest that Oryzalin has a simultaneous, but smaller influence on radicle development. Oryzalin acts by disrupting microtubule formation that induces polyploidy, potentially increasing cell size and morphologically affecting organ growth through mitotic inhibition that can result in impaired cell division and chromosome duplication. This disruption can cause seeds to fail to complete the cell division cycle required for growth, resulting in lower germination capacity [8]. The chance of chromosome set changes or chromosome duplication is influenced by the level of oryzalin that is sufficiently absorbed by the seed, but at high concentrations this mutagen can be lethal [9]. Oryzalin showed a higher percentage of abnormal sprouts than those treated with EMS, this abnormality is most likely due to the effects of polyploidization that occurs as a result of Oryzalin treatment, confirming that polyploidization induced by oryzalin tends to disrupt plant morphological development more significantly than point mutations induced by EMS.

Both EMS and Oryzalin that can inhibit and reduce germination can be related to the difference in water potential. Higher concentrations of chemical mutagens can reduce the water potential outside the seed, so that the seed cannot imbibe optimal water during the germination process [10].

In the control treatment, both of Ratna flowers genotypes (purple and pink) showed the same cotyledon color of purple, indicating that under normal conditions, the genotype of Ratna flowers from Sibanggede genetically has a dominant character in expressing anthocyanin pigments in the cotyledon tissue to produce purple color. Cotyledon color changes observed after EMS and Oryzalin treatment indicate that the both mutagens interfere with pigment biosynthesis pathways. EMS treatment led to a lighter purple cotyledon in the purple genotype and a light yellowish-purple color in the pink genotype, indicating a decrease in anthocyanin intensity. The pink genotype experienced a change in cotyledon color to light yellowish purple. This color change is thought to be the result of mutations in the anthocyanin and carotenoid biosynthetic pathways, forming a combination of pigments that produce more complex coloration. The use of mutagen materials can reduce the production or activity of enzymes such as Chalcone Isomerase that play a role in pigment biosynthesis, so that the resulting color becomes paler [11]. Seedlings grown and derived from EMS treatment also showed symptoms of stunted growth such as small leaves, cup-like curved shape, and paler stem color. These phenomena indicate a disruption in cell division and elongation and a decrease in chlorophyll index due to the mutation, as also found by [12] in potato leaves.

Oryzalin treatment causes changes in cotyledon color to dark purple in both genotypes, this change associated to polyploidization, resulting in different phenotypes including darker sprout color due to the accumulation of anthocyanin pigments in the tissue because polyploid cells tend to have a larger volume and can store more pigments [13]. Oryzalin seedlings had larger and thicker leaves and stems than the control, but with slower growth than EMS. This symptom is typical of the results of polyploidization, where the size of vegetative organs increases due to the double number of chromosomes, but the speed of development decreases [14]. The reduction in growth rate is consistent with findings from studies on *Acacia crassicarpa* treated with increasing concentrations of mutagens [15]. Ratna flower plants appear to have a specific sensitivity to mutations in genes that regulate pigment biosynthesis, minor genetic changes can express different sprout colors. The results showed that the purple genotype had better adaptability to mutagen treatment compared to the pink genotype, indicated by the more optimal plumule and radicle length and fewer abnormal sprouts in the purple genotype. Presumably, the purple genotype has higher genetic stability or more active expression of defense genes against mutagenic stress. Based on the comparison results, EMS showed higher effectiveness in affecting early plant morphological and physiological parameters. Although EMS tends to inhibit growth to some extent, it generated more pronounced and measurable phenotypic diversity than Oryzalin, which primarily induced changes in enlarging the size of vegetative organs without increasing growth rate.

CONCLUSIONS

This study demonstrates that chemical mutagen treatments significantly effect the morphology and early physiological characteristics of Ratna flower plants. EMS chemical mutagens and purple genotype ratna flower showed their effectiveness in producing more phenotypic variation in the early growth or germination phase. Oryzalin treatment is able to show indications of polyploidization which causes greater organ growth. The observed changes in cotyledon color in both treatments indicate that EMS and Oryzalin influence pigment biosynthesis pathway through disruption of gene expression. Further research can be conducted with a more robust methodology to develop these findings and find the right dose of mutagens to produce useful mutant plants.

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