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Research Note

Standardization of EMS doses for mutagenesis in strawberry (*Fragaria x Ananassa* Duch) cv. winter dawn

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Abstract
Induced mutation by Ethyl Methane Sulphonate (EMS) has been found to be a very useful technique for crop improvement. Chemical mutagens, such as EMS, have been widely used to induce a variety of practical variations in several fruit crops. Therefore, a study was undertaken to determine the LD_{50} and the effects of mutagens on growth-related, yield-related, and quality-related observations, as well as the mortality percentage of runners derived from the Winter Dawn variety of strawberry using EMS to create variability for desirable traits. The runners were treated with EMS. The treated runners, along with a control group, were planted for growth in pots under a polytunnel. Based on the probit curve derived from the survival of the treated plant, the LD_{50} dose for EMS was found to be 3%. The 0.1% and 0.2% EMS doses showed good results in terms of growth, yield, and quality. An increase in the concentration of EMS resulted in a decrease in plant survival rate.

Keywords: EMS, Chemical mutagen, strawberry

Strawberries (*Fragaria* × *ananassa* Duch) belong to the Rosaceae family and are recognized as a vital plant species that yield essential fruit. They are cultivated globally due to their nutritional richness, containing high levels of vitamin C, flavonoids, ellagic acid, and anthocyanin (Palei *et al.,*2015).

The strawberry (*Fragaria* × *ananassa* Duch.) is considered one of the most delightful, invigorating, and tender fruits globally. Its extensive genetic diversity, pronounced heterozygosity, and adaptability to various environmental conditions make it the most widely cultivated fruit crop worldwide. The cultivation of strawberries has surged in popularity in Asian nations due to its exceptional yield per square foot in a short period. In India, commercial strawberry cultivation is prominent in states such as Himachal Pradesh, Western Uttar Pradesh, Uttarakhand, Haryana, and Punjab. Approximately 15,600 units of land are dedicated to strawberry farming in total (Cosme *et al*., 2022).

The cultivation of European strawberry species in Western Europe began in the 15th century, marking the origins of strawberry breeding through careful selection and cultivation practices, which were later replicated in Chile. Today, the most widely favored type of strawberry is the garden strawberry, created through the hybridization of two distinct species known scientifically as *Fragaria ananassa*. Despite the prominence of this variety, there exist numerous other strawberry types, some of which are cultivated to varying degrees. Strawberry species are classified into different genetic groups based on their chromosome count. Throughout history, strawberry farmers have employed various breeding methods, starting with traditional plant breeding and advancing to molecular breeding and genetic modification in the 20th

century (Kaushik *et al.,* 2023). EMS stands out as a practical and widely used mutagen for developing mutant populations. Significant progress has been achieved in generating mutants and identifying crucial regulatory genes related to strawberry traits.

Ethyl Methane Sulphonate (EMS) is a commonly used chemical mutagen mentioned by (Ahloowalia and Maluszynski, 2001), which leads to the generation of various morphological variations, as demonstrated by (Lamikaynak, 2016) through EMS treatments. Moreover, EMS has found widespread application across various fruit plant species, including Mango (Sanchez *et al*., 2024), Banana (Liu *et al*., 2024), and Papaya (Ravi *et al*., 2023; (Bhat *et al*., 2017). In this research, strawberry runners were exposed to suitable doses of the chemical mutagen EMS for a duration of 1 hour, with subsequent observations made on growth, yield, and quality post-treatment.

The research was conducted at the Horticulture Research Farm, Lovely Professional University, Phagwara, Punjab, under a protected polytunnel structure during the 2023-2024 growth season. Strawberry cv. Winter Dawn plants were selected for the present investigation and were treated with the chemical mutagen EMS.

Ethyl methane sulphonate treatment : To prepare EMS solutions of 0.1%, 0.2%, and 0.3% concentration, 20 ml of DMSO was used as a carrier agent for each of the three EMS concentrations, dissolved in 979 ml, 978 ml, and 977 ml of distilled water, respectively, with 1 ml, 2 ml, and 3 ml of EMS.

Strawberry runners were soaked in three different doses (0.1%, 0.2%, and 0.3%) of EMS for 1 hour (**Fig. 1**) and immediately transplanted thereafter. Each treatment was replicated three times, with five plants per replication. After treatment, strawberry runners were differentiated based on the different doses, with 25 plants per dose, totalling 100 plants across five replications, which were then transferred to plastic pots for further research.

Probit analysis: The LD_{50} dose is of great importance for understanding the sensitivity of various genotypes to critical doses of mutagens that cause 50 percent mortality. The LD_{50} value for EMS was calculated using probit analysis (Finney, 1964). The probit function is the inverse of the cumulative distribution function (CDF) or quantile function associated with the standard normal distribution.

Impact of ems doses on growth-related observations: The height of each plant in every pot was measured using a meter scale, followed by the calculation of the average height. Additionally, we determined the average leaf count by counting the leaves on each plant in every pot. A Leaf Area Meter was used to measure the size of mature leaves in millimeters. The chlorophyll content in the leaves was measured using a SPAD meter without harming the plants. Observations of days to first flowering and first fruiting were observed in the transplanted plants.

Impact of ems doses yield related and quality attributes observation: The number of flower buds, open flowers, and mature fruits per plant in each pot, along with the total leaf count per plant, was tallied. These counts were then averaged to assess fruit quality. Measurements of fruit length and diameter in millimeters (mm) were recorded using the Digital Caliper-515 (DC-515), and the average measurements for each treatment were calculated. To determine brix percentages, a portable digital refractometer (ERMA, Tokyo) was used.

Statistical analysis: Opstat software was used for data analysis. A one-way ANOVA was conducted to assess the significance of differences among treatments across

Fig. 1. Chemical mutagen doses (EMS) for strawberry runners winter Dawn variety.

various observed parameters. The analysis employed the least significant difference (LSD) test to determine the statistical significance of mean differences, with a significance level of 5%. Treatment effects were evaluated for statistical significance at the 5% level.

Determination of ld_{50} dose for runners : The results obtained from EMS treatments showed a gradual decrease in the survivability of strawberry runners with increasing doses of the mutagenic agent. This trend is consistent with findings from other researchers (Coban, 1998; Sutarto, 2009). Similarly, (Priyadharshni *et al.,* 2020) reported that the mutagenic effectiveness and efficiency of the chemical mutagen EMS in cowpea (*Vigna unguiculata*) decreased as the dosage increased. (Usharani and Kumar, 2015) also noted that the efficiency of EMS was highest at lower and intermediate concentrations of the mutagenic treatment. A similar result was also reported by (Kavithamani *et al.,*2008). The LD_{50} for EMS was calculated based on the survival percentage of treated strawberry runners compared to that of untreated runners **(Table 1)**. The highest percentage of mortality was recorded with 3% EMS (40%), while the lowest was observed with 2% EMS treatment (16%) **(Table 1)**. There was a significant reduction in the sprouting percentage of strawberry runners as the mutagenic dose increased, as shown in **(Table 1)**. In general, lower and moderate doses of EMS gave strawberry runners a better chance of survival than higher dosages. Mutagenic sensitivity depends on the source of irradiation, the dose rate, and the plant material (Spencer-Lopes, 2018). The reason for low sprouting in the present study on mutagenic treatments may be due to postponement in the initiation of mitosis and chromosomal abnormalities induced by activation of enzymes (Bind and Dwivedi, 2014).

Impact of ems mutagen on growth attributes: The strawberry plants treated with EMS showed favorable growth traits, including maximum plant height, more leaves, larger leaf area, and higher chlorophyll content. When comparing different doses of EMS treatment in these plants, it was found that a 2% EMS dose resulted in the tallest plants, while a 3% EMS dose led to the shortest ones. In contrast, plants in the control group, which were not treated, were consistently shorter compared to all EMS-treated plants **(Table 2)**. A study on cluster bean species by (Mullainathan and Arulbalachandran, 2012) similarly demonstrated that increasing EMS doses resulted in reduced leaf number and plant height. Notably, plant height varied significantly with different EMS concentrations, with higher doses leading to reduced plant height compared to untreated plants, as noted by (Shailendra *et al*.,2010). These findings align with earlier research, such as that of (Jabeen and Mirza, 2004) on *Capsicum annuum*, in mango by (Rime *et al.,*2019) , in sunflower by (Cvejic *et al.,*2011). This phenomenon may be attributed to abnormal cell division, potentially affecting gene expression (Tarigan *et al.,*2021).

It was observed that plants treated with various doses of EMS had varying leaf numbers. The highest leaf count was observed in plants treated with a 2% EMS dose, followed by those treated with a 1% EMS. The lowest leaf count was observed in plants treated with a 3% EMS dose. Comparatively, the control group had the fewest leaves **(Table 2)**. In the Camarosa variety of strawberry, a 0.1% EMS concentration resulted in the highest number of leaves per plant, which was the best among all treatments according to (Bhat *et al.,*2017). However, (Ravi *et al.,* 2023) reported that in the Papaya variety CO7, the maximum number of leaves was observed at a 0.2% EMS concentration. In chrysanthemum var. Poornima white, the maximum number of leaves was recorded at the 2% EMS dose, with the minimum at the highest dose of 5% EMS (Anitha *et al.,* 2021). In the present study, the Leaf Area Index (LAI) varied among the mutagen-treated strawberry plants, with the maximum LAI at the 2% EMS and the minimum at the 3% EMS dose, while in the untreated plants it was the lowest LAI **(Table 2)**. Similar trend was noticed in Kinnow mandarin by (Kumar *et al*., 2021) and in *Abelmoschus esculentus* by (Deepthi *et al.,*2016). With respect to Chlorophyll content, it was observed to be highest at 2% EMS dose, followed by the 1% EMS dose, and lowest at the 3% EMS dose **(Table 2)**. The leaf chlorophyll content decreased as the EMS dose increased. This is similar to the findings by (Akhtar, 2014) and (Deepthi *et al.,*2016) in *Abelmoschus esculentus*.

Impact of ems mutagen on yielding attributes: The strawberry runners treated with different doses of EMS showed varied initiation times for the first bud. Plants treated with 1% and 2% EMS doses exhibited early bud emergence, while untreated plants showed late bud emergence **(Table 3)**. Similar earliness in flower bud initiation was observed in chrysanthemum plants treated with a 2% EMS dose by (Anitha *et al.,*2021). In the present study, it was noted that the time taken for fruit set was shortest in plants treated with 1% EMS dose,

Table 1. Various computations for probit line LD_{50}.

Conc.	Total No.	No. Kills	%Mort	Log (Dose)	Exp. Prop	Emp Probit	Exp Probit	Work. probit
1% EMS	25	6	24	0.000	0.038	-1.776	-1.776	-1.687
2% EMS	25	4	16	0.301	0.162	-1.097	-0.986	-1.091
3% EMS	25	10	40	0.477	0.300	0.473	-0.523	-0.472
Control	25	7	28	0.528	0.139	0.249	-0.423	-2.343

Table 2. Effect of EMS on growth-related observation in strawberry cv Winter Dawn.

Dosage EMS	Plant Height	Leaf Number	LAI (mm)	Leaf Chlorophyll
1%	23.98^{b} (4.99)	13.85^{a} (3.85)	22.27^{b} (4.87)	52.41^{b} (7.26)
2%	26.28^{a} (5.22)	14.3^{a} (3.91)	24.1^{a} (5.01)	59.28^{a} (7.76)
3%	22.15^{c} (4.81)	12.62^{b} (3.69)	23.04^{ab} (4.90)	51.82^{b} (7.30)
Control	19.46^{d} (4.52)	12.21^{b} (3.64)	22.79^{b} (4.82)	52.10^{b} (7.28)
C.D.	1.051 (0.106)	0.959 (0.127)	1.184 (0.121)	1.87 (0.127)
SE(m)	0.337 (0.034)	0.308 (0.041)	0.38 (0.039)	0.6 (0.041)
SE(d)	0.477 (0.048)	0.436 (0.058)	0.537 (0.055)	0.849 (0.058)
C.V	2.937 (1.392)	4.649 (2.161)	3.297 (1.588)	2.227 (1.101)

The values shown in the table represent the average of five replications. Distinct letters within the same column indicate significant variances at a significance level of $P < 0.05$, determined by the Ducan Multiple Range Test. Values in the brackets are transformed.

Table 3. Effect of EMS on fruit-related observation in strawberry cv Winter Dawn

Dosage EMS	Days to Bud	Number of Bud	Days to flower	Number of flowers
1%	53.1^{b} (7.33)	31.33^{b} (5.68)	68.4^{b} (8.33)	31.44^{b} (5.69)
2%	53.4^{b} (7.37)	33.45^{a} (5.86)	65.4^{c} (8.14)	34.33^{a} (5.99)
3%	58.9^{a} (7.74)	30.87^{b} (5.64)	70.1^{a} (8.43)	30.36^{b} (5.68)
Control	60.4^{a} (7.83)	30.6^{b} (5.62)	71.4^{a} (8.51)	31.3^{b} (5.68)
C.D.	1.611 (0.107)	1.376 (0.12)	1.424 (0.085)	0.428 (0.116)
SE(m)	0.517 (0.034)	0.442 (0.039)	0.457 (0.027)	0.428 (0.037)
SE(d)	0.731 (0.048)	0.625 (0.055)	0.646 (0.039)	0.605 (0.053)
C.V	1.831 (0.904)	2.799 (1.356)	1.328 (0.656)	2.664 (1.295)

The values shown in the table represent the average of five replications. Distinct letters within the same column indicate significant variances at a significance level of $P < 0.05$, determined by the Ducan Multiple Range Test. Values in the brackets are transformed.

followed by those treated with 2% EMS dose. Control and the dose 3% EMS were observed to be later to reach fruit set. Similarly, in the Camarosa strawberry variety, plants treated with a 1% EMS dose showed early fruit set, as reported by (Bhat *et al.,* 2017). It was observed that the maximum number of buds was observed in plants treated with a 2% EMS dose, followed by those treated with a 1% EMS dose. Control plants had the fewest buds, and plants treated with a 3% EMS dose also had a lower bud count **(Table 3).**

With respect to the fully opened flowers, the highest number was recorded in the 2% EMS dose, followed by the 1% EMS dose, while the lowest number of flowers was recorded in the 3% EMS dose and untreated plants. The maximum number of fruits was observed in the 2% EMS dose, followed by the 1% EMS dose, with the minimum number of fruits in the control plants and the 3% EMS dose **(Table 4)**.

Impact of ems mutagen on quality attributes: It was observed that plants treated with different doses of EMS showed distinct fruit characteristics. Those treated with a 2% dose exhibited the longest and widest fruits compared to other treatments. The control group, which received no treatment, had the shortest and narrowest fruits. In terms of fruit weight, plants treated with a 2% EMS dose bore the heaviest fruits, followed by those treated with a 1% EMS dose. The control and the plants treated with 3% EMS dose had the lightest fruits. Overall, consistently higher fruit weight was observed in plants treated with a 2% EMS dose, whereas lower weights were seen in untreated plants or those treated with a 3% EMS dose. EMS, a chemical mutagen, can be used to improve desired characteristics such as yield-related traits (Botticella *et al.,* 2011). Highest number of fruits per plant was observed in plants treated with 2% EMS followed by 1%, while the lowest fruit yield was observed in the control and then in the 3% EMS dose **(Table 4)**. Similar results were observed in the Camarosa strawberry variety by (Bhat *et al.,* 2017), where 0.1% EMS produced the highest fruit yield per plant, followed by 0.2%, and the lowest yield was in the 0.3% EMS dose .

The application of a low dose of the chemical mutagen EMS has shown promising results in inducing specific mutations while minimizing damage to the plant's genetic material. This targeted mutagenesis approach can lead to the development of desirable traits such as improved plant characteristics, increased yield potential,

Table 4. Effect of EMS on yield-related observations in strawberry cv Winter Dawn

Dosage EMS	Days to fruit set	Number of fruits	Fruit Length	Fruit Diameter	Fruit weight	Fruit Yield (gm) per plat
1%	87.4^{c} (9.40)	33.60^{b} (5.796)	39.49^{ab} (6.36)	28.20^{ba} (5.28)	12.87^{b} (3.15)	428.5^{b} (20.17)
2%	88.8^{bc} (9.47)	35.54^{a} (6.045)	40.49^{a} (6.44)	30.32^{b} (5.36)	13.47^{a} (3.80)	536.5^{a} (23.16)
3%	90.3^{ab} (9.55)	32.19^{b} (5.761)	38.38^{b} (6.27)	27.35^{c} (5.19)	11.34^{b} (3.60)	425^{b} (20.61)
Control	91.2^{a} (9.60)	31.95^{b} (5.74)	35.60^{c} (6.11)	26.98^{c} (5.12)	11.17^{b} (3.48)	403.7^{b} (20.11)
C.D.	1.883(0.099)	1.453 (0.123)	1.325 (0.106)	1.274 (0.089)	0.971 (0.134)	64.3 (1.187)
SE(m)	0.605 (0.032)	0.466 (0.039)	0.425 (0.034)	0.409 (0.028)	0.321 (0.043)	20.639 (0.477)
SE(d)	0.855 (0.045)	0.659 (0.056)	0.601 (0.048)	0.578 (0.04)	0.441 (0.061)	29.188 (0.675)
C.V	1.352 (0.67)	2.82 (1.351)	2.21 (1.079)	2.898 (1.0844)	5.196 (2.385)	9.205 (4.513)

The values shown in the table represent the average of five replications. Distinct letters within the same column indicate significant variances at a significance level of P < 0.05, determined by the Ducan Multiple Range Test. Values in the brackets are transformed.

or modified growth patterns. The efficacy of a low EMS dose lies in its ability to selectively target specific genes or genetic regions, reducing the occurrence of random mutations that could be detrimental to the overall health and development of the plant. Moreover, lower doses of EMS are less likely to trigger multiple trait responses in plants, allowing for smoother recovery and continued growth with minimal negative consequences. Among the various concentrations tested, the 0.2% EMS dose demonstrated the most favourable outcomes, followed by the 0.1% dose, while the 0.3% EMS dose and the control group exhibited comparatively lesser effects on inducing desirable mutations and traits.

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