

The Effect of Different Doses of the Chemical Mutagen EMS on Morphological, Physiological, Productivity, and Quality Traits of the Soybean Genotype Sb 239 (*Glycine max* L. Merr.)

Dr. Yara Amer Zarba* 

(Received 16 / 4 / 2026. Accepted 24 / 6 / 2026)

□ ABSTRACT □

Genotypic soybean seeds of the *Glycine max* Sb 239 variety, obtained from the Agricultural Scientific Research Authority in Damascus, were subjected to mutagenesis using the chemical mutagen EMS (ethyl methane sulfonate) at concentrations of 0.1, 0.2, 0.3, and 0.4) % v/v. This study aimed to determine the optimal EMS concentration and its effect on certain morphological, physiological, yield, and quality traits of the soybean crop. The objective is to develop new varieties with high yield. Early flowering and maturity for use in subsequent breeding programs. The mutated seeds were planted in permanent fields in the Damsarkho area of Latakia during the 2024–2025 growing season. By designing completely randomized blocks with three replicates in the second generation, the descriptive statistical analysis and variance were performed using appropriate statistical software (SPSS20).

The results indicated that the chemical mutagen EMS is effective in inducing mutations in soybeans across the concentrations studied, with a concentration of 0.1% v/v being the most effective for most of traits studied. A gradual decrease in the values of most traits was observed as treatment concentrations increased (0.2, 0.3, and 0.4)% v/v, particularly in yield, yield components, and protein content in the selected plants.

Keywords: Different Doses· Chemical Mutagen EMS· Soybean Genotype Sb 239

Copyright



:Latakia University journal (formerly Tishreen) -Syria, The authors retain the copyright under a CC BY-NC-SA 04

* Researcher- PhD in Agricultural Engineering- Latakia University(formerly Tishreen)- Latakia-Syria nanoyaro55@gmail.com

تأثير جرعات مختلفة من المطفر الكيميائي EMS على بعض الصفات المورفولوجية والفيزيولوجية والإنتاجية والنوعية للطرار الوراثي Sb 239 من فول الصويا *Glycine max* (L.) Merr.

د. يارا عامر زربا^{*}

(تاريخ الإيداع 16 / 4 / 2026. قبل للنشر في 24 / 6 / 2026)

□ ملخص □

عرضت بذور الطراز الوراثي من فول الصويا *Glycine max* Sb 239 المستقدمة من هيئة البحوث العلمية الزراعية في دمشق، للتطعيم باستخدام المطفر الكيميائي EMS (ethyl methane sulfonate) بتركيز (0.1، 0.2، 0.3 و 0.4) % v/v، وذلك بهدف تحديد التركيز الأمثل لجرعات EMS وتأثيره على بعض الصفات المورفولوجية والفيزيولوجية والإنتاجية والنوعية لمحصول فول الصويا من أجل الوصول إلى طرز جديدة ذات إنتاجية عالية وذات صفتي باكورية في الإزهار والنضج للاستخدام في برامج التربية اللاحقة. زرعت البذور المطفورة في الأرض الدائمة بمنطقة دمسرخو ضمن مدينة اللاذقية خلال الموسم الزراعي 2024-2025. بتصميم القطاعات العشوائية الكاملة وبثلاث مكررات في الجيل الثاني، كما أجري التحليل الإحصائي الوصفي (Description) والتباين باستخدام البرامج الإحصائية المناسبة (Excel) و (SPSS20).

أشارت النتائج إلى وجود طفرات مستحدثة أثرت على العديد من الصفات الزراعية، وأن المطفر الكيميائي EMS فعال في إحداث الطفرات في نبات فول الصويا مع اختلاف التراكيز المدروسة، وكان التركيز v/v 0.1% أفضل التراكيز لمعظم الصفات المدروسة مع حدوث الانخفاض التدريجي لقيم معظم الصفات مع ازدياد التراكيز المعاملة (0.2، 0.3 و 0.4) % v/v وخاصة في الإنتاجية وعناصرها ونسبة البروتين عند النباتات المنتخبة.

الكلمات المفتاحية: جرعات مختلفة، مادة مطفرة كيميائية EMS، الطراز الوراثي لفول الصويا Sb 239

حقوق النشر : مجلة جامعة اللاذقية (تشرين سابقاً) - سورية، يحتفظ المؤلفون بحقوق النشر بموجب
الترخيص CC BY-NC-SA 04

^{*} باحث - دكتوراه في الهندسة الزراعية جامعة اللاذقية (تشرين سابقاً) - اللاذقية - سوريا. nanoyaro55@gmail.com

Introduction:

The soybean crop (*Glycine max* [L.] Merr.) belongs to the Leguminosae family and is a self-pollinating crop (2N=40), with a cross-pollination rate of approximately 1–2% [5].

Soybeans are considered to be among the world's leading oil crops, accounting for approximately 57% of the vegetable oils produced worldwide [27].

Soybeans are a multipurpose crop, cultivated for edible oils, industrial use, human food, livestock feed, and as a source of bioenergy [1].

The Syrian Arab Republic is confronted with a multitude of economic challenges in the agricultural sector, including the procurement of vegetable oil and animal feed for the local market. This has resulted in the state incurring significant expenditure to import these products from foreign countries, leading to increased costs for oil, various meat products, eggs, and other goods.

Consequently, there is a growing interest in field crops, particularly legumes, to address this significant shortfall, with soybeans being a primary focus due to their nutritional, industrial, and feed value, as well as their capacity to enhance agricultural soil properties through atmospheric nitrogen fixation.

Consequently, there is a necessity to employ advanced scientific methodologies for the purpose of crop breeding and enhancement, with the objective of cultivating varieties that exhibit favorable agronomic characteristics. This can be achieved through conventional breeding techniques [2] and the refinement of agricultural practices, in addition to the integration of contemporary methodologies such as the utilisation of mutagens, encompassing physical and chemical mutagens, or a combination thereof [17].

Chemical and physical mutagens are typically utilised at low concentrations (doses) in plant breeding programmes obtaining new varieties that exhibit high productivity and desirable characteristics such as early maturity, short plant height, and disease resistance [7], in addition to increased oil and protein content [19].

It is imperative to ascertain the stability of mutations across multiple generations and under various environmental conditions. The escalating global demand for food, in terms of both quality and quantity, has precipitated the development of high-yielding plant varieties, principally through the utilisation of diverse breeding methodologies, including chemical mutagenesis using EMS.

EMS (ethyl methane sulfonate) is a DNA-acting agent [23] that exerts a marked effect on the chemical composition of seeds, including those of soybeans, thus enabling the acquisition of desirable traits [28]. EMS has also been used to obtain high-yielding soybean varieties [26]. The mechanism by which EMS affects soybeans is multi-layered, beginning with its interaction with DNA and culminating in the emergence of new heritable traits. This occurs through EMS entering the plant cell and performing an alkylation process, which involves adding an ethyl chemical group to the nitrogenous bases of DNA, most notably guanine (G) [29].

A plethora of mutation induction methods have been employed in the study of soybean seeds, with a range of traits (morphological, physiological, chemical, and yield-related) being examined to validate the occurrence of mutations [14, 10].

Soybeans are an oilseed and legume crop of high nutritional and economic value. Due to low domestic production coupled with increasing demand for soybean seeds as a food, industrial, and animal feed crop, breeding methods to increase productivity have become essential. However, the limited genetic diversity of cultivated varieties restricts their improvement using traditional methods. Therefore, induced mutations are an effective

tool for creating new variations in morphological, physiological, and genetic traits. Ethyl methanesulfonate (EMS) is widely used as a potent chemical mutagen. The present study aspires to ascertain the optimal concentration of the chemical mutagen EMS (ethyl methane sulfonate) and its impact on specific morphological, physiological, productive, and quality traits of the soybean crop. The objective is to cultivate the most superior variety, characterised by elevated yield and early flowering and maturity.

Research Materials and Methods:

-Plant material and its source:

The Genotype Sb239 of soybeans, sourced from the General Organization for Agricultural Scientific Research in Damascus, was utilised in the study. The following characteristics are indicative of this phenomenon:

Table 1: Features of the Sb239 genotype

genotype	Germination rate%	Number of days until flowering	Number of days until ripening	Average plant height (cm)	Weight of 100 seeds (g)	Seed yield kg/ha
Sb239	90	49	118	58	17.3	2564

-Time and place of the study:

The mutant seeds were planted on 27 May 2024, during the first growing season, and the seeds of the selected plants from the first generation were planted on 26 May 2025, during the second growing season. These seeds were planted at a private farm in the Damsarkho area of Latakia Governorate and at the Scientific Research Laboratory of the Faculty of Agricultural Engineering at the University of Latakia.

mutating

The genetic mutagenesis of soybean seeds was performed using the EMS mutagen, as outlined in [28], with certain modifications, to obtain the first mutant generation (M1).

The seeds were subjected to an initial soaking in water for a period of four hours, with the objective of activating the seeds. Following this, the seeds were then air-dried. In order to conduct this experiment, 300 seeds were placed within a 1000-ml bottle, to which 400 ml of distilled water was added. Fifteen bottles were prepared and placed on a shaker (JSR, JSOS 500) at a speed of 150 rpm for 4 hours at a temperature of 25°C. Subsequently, the seeds were washed with water in order to remove any extraneous impurities.

Four concentrations of EMS (Sigma) were prepared using distilled water (0.1, 0.2, 0.3, and 0.4) % v/v (LD50 = 0.71% according to a previous study conducted under the same conditions) and added to the bottles (1 mL per seed), with four bottles per concentration (approximately 1,000 seeds), plus three bottles for the control (distilled water only).

The bottles were placed on a shaker at a speed of 150 revolutions per minute for a period of 4 hours at a temperature of 25 degrees Celsius. The seeds were then subjected to a washing process with tap water, repeated ten times.

The EMS solution was placed in a 4% sodium hydroxide solution for 24 hours at 25°C [9]. It is noteworthy that the seeds were immersed in the mutagen for a duration of 24 hours, in addition to the control group, which did not undergo such treatment.

Cultivation method:

First generation:

The experiment was conducted in the first generation, with each treatment planted in a separate experimental plot measuring 2 x 2 m. Each plot was subdivided into five rows, with a spacing of 50 cm between rows and 10 cm between plants within each row.

Service aisles measuring 50 centimetres in width were left between adjacent experimental plots. At the conclusion of the initial season, plants that exhibited positive phenological, morphological, or yield changes in comparison with the control in the first generation (M1) were selected and preserved individually (each plant separately) for planting the following year, to obtain M2 Mutant. It is important to note that the selection of all M1 plants was conducted when the number of remaining plants was minimal, a consequence of the treatments applied.

Second Generation:

In the subsequent generation, seeds from the plants selected in the first generation were sown in triplicate using a single-row planting method to produce M2 plants. The spacing between rows was 50 cm, with each row measuring 1 m in length, and a rate of 20 seeds per row was employed. The plants of each treatment were planted independently in order to distinguish them from the other treatments (so that each row represented an independent Genotype), in addition to planting three rows of the control for each treatment for comparison. Selection was also applied to the mutant plants, in which genetic segregation began to appear at the end of the year, with only 5–10% of the M2 plants selected (since most of the mutations were undesirable), based on the best morphological and productive traits for cultivation in the third season to obtain M3 plants.

Service operations after planting:

In the initial phase, irrigation was administered on a weekly basis; subsequently, this was reduced to a fortnightly schedule. A complete fertiliser (Fertium) containing iron (N: P: K: Fe, 20:20:20:5) was added to the soil. The application of an insecticide (Dolan) was followed by a manual removal of weeds. First-generation (M1) plants were obtained and properly preserved for cultivation in the second generation.

The cultivation conditions for the second generation were comparable to those of the first generation, in terms of planting method, irrigation frequency, fertilization, pesticides, and agricultural practices. First-generation plants were permitted to self-pollinate, thus yielding second-generation individuals. Following the processes of pod formation and desiccation, the second-generation (M2) seeds were collected, and the seeds from each concentration were subsequently amalgamated into a single bag for subsequent testing.

-Environmental conditions:

The soil at the research site (Table 2) is characterised by a clay-loam texture and a slightly alkaline pH. The soil is distinguished by a high total carbonate content, low salinity, and high levels of available phosphorus, with moderate potassium content, rendering it conducive to the growth and development of soybean crops.

Table (2) presents the results of a soil analysis of the experimental site.

Depth (cm)	Physical analysis			absorbable elements			Chemical analysis			
	%sand	%Salt	%clay	N PPM metallic	P PPM	K PPM	N%	CaCO 3%	EC 5:1 (Millimos/c m)	pH 5:1
30-0	18	38	44	5.6	21.5	182	0.16	31.6	0.28	7.1
Description	-	-	Clay Celtic	poor	Rich	Medium	Medium	High	few	mild base

Weather data were monitored and recorded through out the study period. Temperatures did not reach the minimum or maximum weeds that would inhibit growth, and were thus suitable for the cultivation and growth of various soybean varieties. However, due to inadequate rainfall, the crops were irrigated multiple times during the study period, at a frequency of approximately one irrigation every ten days, contingent on the air temperature.(Table 3)

Table (3) presents the prevailing climatic conditions at the study site during the 2025 season.

month	Rainfall (mm)	Temperature (degrees Celsius)		
		Great	minor	average
April	24	25.7	17.3	21.5
May	27	27.1	18.4	22.75
June	18	28.9	21.4	25.15
July	0	31.4	24.5	27.95
August	0	33.9	27.8	30.85

Latakia Meteorological Station (Bouqa).

Studied characteristics readings:

1-Plant height (cm): (Taken at the end of the growth period (pod formation) from the point of contact of the plant with the ground surface to the tip of the plant's crown).

2-Number of branches.

3-Number of plant leaves.

4-Leaf area on a single plant (cm²):

Area of a single leaf = Leaf length x Leaf width x Correction factor (for beans) 0.583

Leaf area = Leaf area x Number of leaflets on the leaf [12].

Plant leaf area = area of a single leaf x number of leaves on the plant [12].

5-Guide to plant leaf area:

Leaf area index = Leaf surface area of the plant / Area it occupies of the ground (800 cm²) [12].

6-Pod length/cm.

7-The weight of the horn(g).

8-Seed weight per pod/g: (This is done by separating the seeds of each pod and recording the weight of the seeds in it).

9-Weight of 100 seeds/g: (The seeds of each plant were counted and weighed, then the weight of 100 seeds was calculated).

10-The number of pods in a plant.

11-Weight of pods per plant (g): (The seeds of each plant were counted and weighed).

12-Weight of seeds per plant/g: (This is done by separating the pods of each plant individually and recording the weight of the seeds in them).

13-Protein percentage: According to Keldahl's method [6].

Percentage of protein = Volume (in ml) of acid consumed for titration x 5.71 x 100 / Sample weight.

Statistical analysis:

A descriptive and variance statistical analysis of the studied traits in the plants selected from each treatment was performed using appropriate statistical software (Excel) and (SPSS20).

Results and Discussion:

Statistical indicators of the effect of the EMS mutagen on the traits studied:

1- Plant height (cm):

All concentrations utilised resulted in a discernible and substantial alteration in plant height for second-generation plants (M2), with genetic variations manifesting as depicted in Table 4. This was most evident at the 0.1% v/v concentration, which had a positive and stimulating effect; many plants were selected, with an average height of 73.45 cm. The remaining concentrations (0.2, 0.3, 0.4% v/v) exhibited a gradual negative effect

on this trait, with rates of (17.04–40.01–48.53%) being observed, respectively, compared to the control, which had a mean of (72.50 cm). The outcomes of this study are in alignment with the observations reported in [16], which documented statistically significant variations in plant height among the various mutant genotypes. Specifically, NARC-2 and AJMERI-2 exhibited plant heights of 26.99 cm and 26.933 cm, respectively. The study concluded that plant height undergoes a decline in proportion to increasing mutant doses.

This finding is consistent with the results reported in [21], which demonstrated that increasing the applied doses resulted in a decrease in plant height in the majority of experiments. The decline in the stature of mutant plants is attributed to divisions caused by mutation and the expression of plant hormone synthesis genes [8].

The coefficient of variation (CV %) exhibited a low value in the control group at 7.35%. However, it demonstrated a higher value and attained a significant level at numerous seed soaking concentrations. The highest recorded value was observed at a concentration of 0.3% v/v, where it reached 30.61%. Additionally, the range was found to be more extensive at all concentrations in comparison to the control group, thereby confirming an increased genetic variation in second-generation plants. The significance of these alterations, resulting from mutagen treatment, lies in their role in the evaluation of mutagen efficacy [20].

It is important to note that plant height has a significant impact on the number and location of pods. Taller crops may be more efficiently harvested, as taller plants may bend over before harvest [25].

Table (4): Statistical indicators of the effect of the EMS mutagen on the plant height trait (cm) of Sb239 (M2) soybean plants

Mutagen concentration V/V %	Number of selected plants	range	Minimum value	Supreme Value	Average quality	Standard error	standard deviation	Contrast	Coefficient of variation
0	11	14.50	72.80	87.30	72.50	1.64	5.44	29.64	7.35
0.1	27	29.57	49.30	78.90	73.45	1.74	9.05	81.96	12.32
0.2	24	35.00	42.83	77.83	60.93	2.43	11.93	142.43	19.57
0.3	13	23.20	31.50	54.70	44.06	3.74	13.49	182.08	30.61
0.4	5	16.40	28.20	44.60	37.80	3.49	7.82	61.35	20.68

2- Number of branches:

Subsequent to the second generation, genetic variations manifested in the plants, giving rise to an increased number of branches in the majority of the plants when compared with the control. This effect was most evident at concentrations of 0.1% and 0.2% (v/v) (3.65–3.57 branches), which exhibited superior performance in comparison to the control group (3.60 branches) (see Table 5 for details). The present findings are consistent with those reported in [13] on the positive role of gamma radiation in increasing the number of branches in plants. It is also noteworthy that the range exhibited by the experimental group exceeded that of the control group at numerous concentrations. This finding is widely regarded as an indicator of increased genetic variation that is conducive to the selection of this particular trait. The coefficient of variation (CV%) ranged from moderate to high in all treatments studied, including the control and the 0.3% v/v concentration, at 36.11%, thus illustrating the effect of the concentrations used in broadening the genetic variation base, which has breeding significance for selection for this trait. [11] Ascribed the augmented branching in plants treated with mutations to accelerated cell division, elongation, and the synthesis of plant proteins or nucleic acids.

Table (5) Statistical indicators of the effect of the EMS mutagen on the branching number trait of Sb239 (M2) soybean plants

Mutagen concentration V/V %	Number of selected plants	range	Minimum value	Supreme Value	Average quality	Standard error	standard deviation	Contrast	Coefficient of variation
0	11	2.00	3.00	5.00	3.60	0.21	0.72	0.53	19.72
0.1	27	2.33	2.00	4.33	3.65	0.13	0.72	0.52	19.69
0.2	24	2.66	2.00	4.66	3.57	0.22	1.09	1.19	30.47
0.3	13	2.66	2.00	4.66	3.43	0.34	1.24	1.54	36.11
0.4	5	2.00	2.00	4.00	3.20	0.44	1.00	1.00	31.25

3-Number of plant leaves.

All mutant concentrations demonstrated a non-significant decrease in leaf number in comparison with the control Table (6). It is noteworthy that the coefficient of variation (CV%) exhibited elevated levels in the majority of the treatments examined, with the maximum value recorded at the 0.3% v/v concentration (26.03%) and the most extensive range observed at the 0.1% v/v concentration (16.60 leaves) in comparison with the control (6.00 leaves). This finding serves to substantiate the existence of substantial genetic variation in second-generation plants, thereby establishing a foundation for the selection of the trait under investigation. The findings of certain studies have indicated that low concentrations have been shown to increase the number of leaves; while conversely, this number has been shown to decrease at high concentrations [26].

Table (6) Statistical indicators of the effect of the EMS mutagen on the number of leaves trait in the plant for soybean plants Sb239 (M2)

Mutagen concentration V/V %	Number of selected plants	range	Minimum value	Supreme Value	Average quality	Standard error	standard deviation	Contrast	Coefficient of variation
0	11	6.00	30.00	36.00	35.41	1.37	4.56	20.90	12.87
0.1	27	16.60	18.00	34.66	28.51	0.88	4.59	21.14	16.09
0.2	24	13.33	16.33	29.33	23.41	0.87	4.27	18.29	18.23
0.3	13	10.00	12.33	22.33	18.13	1.30	4.72	22.32	26.03
0.4	5	7.00	15.00	22.00	18.50	1.68	3.76	14.00	20.32

4-Leaf area on a single plant (cm²):

Multitudes of variations in leaf area were observed among individual plants in the second generation of mutants in comparison to the control group. The most pronounced difference was observed at a concentration of 0.1% (v/v) (1975.77 cm²) in comparison to the control (2852.25 cm²). This finding facilitated the selection of several distinctive plants due to their positive and stimulating effect. The concentration of 0.3% v/v exerted a deleterious effect on this trait, as evidenced by the lowest recorded value of 1063.91 cm² at this concentration.

It is observed that the coefficient of variation (CV%) exhibited elevated levels at the majority of concentrations, with the maximum value recorded at the (0.3% v/v) concentration (48.30%), and the range demonstrated its widest extent at the (0.1% v/v) (1841.53 cm²). This is in comparison to the control (2852.26 cm²), thereby confirming the presence of augmented genetic variation in certain second-generation plants. This provides a foundation for the selection of plants with regard to the studied trait. The increase in the number of pods and seeds may be attributed to the increased leaf area, which is a productive trait [3].(Table 7).

Table (7): Statistical indicators of the effect of the EMS mutagen on the leaf area trait of Sb239 (M2) soybean plants

Mutagen concentration V/V %	Number of selected plants	range	Minimum value	Supreme Value	Average quality	Standard error	standard deviation	Contrast	Coefficient of variation
0	11	2852.26	1322.24	4174.51	2852.25	27.098	89.85	8073.50	3.15
0.1	27	1841.53	1030.74	2872.27	1975.77	101.17	525.72	276384.50	26.60
0.2	24	1580.51	745.65	2326.16	1417.92	120.40	589.86	347934.90	41.60
0.3	13	1139.47	482.95	1655.42	1063.91	142.54	513.94	264139.80	48.30
0.4	5	632.26	839.52	1471.78	1107.40	147.59	330.03	108921.51	29.80

5- Guide to plant leaf area:

As demonstrated in Table 8, the most significant genetic segregation in the leaf area index trait was observed at a concentration of 0.1% v/v (2.46), in comparison to the control (3.56). This is attributable to its positive and stimulatory effect on selection. The concentrations (0.3% v/v) and (0.4% v/v) exerted a negative effect on this trait, with values of (1.32) and (1.38), respectively.

It is noteworthy that the highest coefficient of variation (CV%) was observed at the concentration of 0.3% v/v (54.70%), and the widest range was observed at the concentration of 0.1% v/v (2.20) compared to the other concentrations, while the control value was (3.56). This confirms increased genetic variation in some second-generation plants, which provides a basis for selection regarding the trait under study.

Table (8): Statistical indicators of the effect of the EMS mutagen on the leaf area index trait of soybean plants Sb239 (M2)

Mutagen concentration V/V %	Number of selected plants	range	Minimum value	Supreme Value	Average quality	Standard error	standard deviation	Contrast	Coefficient of variation
0	11	3.56	1.65	5.12	3.56	0.33	1.12	1.26	31.53
0.1	27	2.20	1.28	3.17	2.46	0.12	0.65	0.42	26.48
0.2	24	1.97	0.93	2.90	1.76	0.15	0.73	0.54	41.59
0.3	13	1.42	0.64	2.06	1.32	0.20	0.72	0.52	54.70
0.4	5	0.79	1.04	1.84	1.38	0.17	0.40	0.16	28.98

6-Pod length (cm):

Pod length is a fundamental trait due to its contribution to seed yield. Table (9) indicates that the most significant concentration for this trait was 0.1% v/v, which had a positive and stimulating effect, resulting in the selection of many superior plants; some of them reached a length of 5.17 cm compared to the control (5.07 cm). The concentrations (0.2% v/v), (0.3% v/v), and (0.4% v/v) harmed this trait, with the lowest value observed at the (0.3% v/v) concentration (4.63 cm). We note that the coefficient of variation (CV%) was highest at the 0.4% v/v concentration (9.25%), and the range was widest at the 0.4% v/v concentration (1.11 cm) compared to the control (0.70 cm). The results for this trait are attributed to the effect of the mutagen at these concentrations, which led to genetic changes.

Table (9) Statistical indicators of the effect of the EMS mutagen on the pod length trait (cm) of Sb239 (M2) soybean plants

Mutagen concentration V/V %	Number of selected plants	range	Minimum value	Supreme Value	Average quality	Standard error	standard deviation	Contrast	Coefficient of variation
0	11	0.70	4.70	5.40	5.07	0.08	0.28	0.08	5.57
0.1	27	0.73	4.60	5.50	5.17	0.04	0.20	0.04	4.02
0.2	24	0.90	4.40	5.30	5.09	0.04	0.20	0.04	3.97
0.3	13	0.46	4.70	5.03	4.63	0.05	0.18	0.03	4.07
0.4	5	1.11	4.00	5.10	4.95	0.20	0.45	0.21	9.25

7-The weight of the horn (g):

The data presented in Table (10) demonstrate the positive impact of the EMS additive on horn weight, particularly at a concentration of 0.1% v/v (0.38 g) in comparison to the control (0.37 g), while the weight of certain horns attained 0.31 g at the higher concentrations. The coefficient of variation (CV%) ranged from low to high, with the highest value recorded at a concentration of 0.2% v/v (23.17%), and the range was widest at the 0.4% v/v concentration (0.12 g), which was equivalent to the control (0.12 g). This finding suggests the presence of genetic variation in this trait, attributable to varying mutagen concentrations. This finding aligns with the outcomes of analogous studies on the impact of radiation on soybean plants, which substantiated the role of mutagens in enhancing pod weight and fostering the emergence of genetic variation [24].

Table (10) Statistical indicators of the effect of the EMS mutagen on the pod weight (g) trait of Sb239 (M2) soybean plants

Mutagen concentration V/V %	Number of selected plants	range	Minimum value	Supreme Value	Average quality	Standard error	standard deviation	Contrast	Coefficient of variation
0	11	0.12	0.33	0.45	0.37	0.01	0.05	0.003	14.80
0.1	27	0.09	0.32	0.41	0.38	0.01	0.08	0.007	23.04
0.2	24	0.06	0.30	0.36	0.34	0.01	0.07	0.006	23.17
0.3	13	0.05	0.27	0.33	0.31	0.01	0.06	0.004	22.03
0.4	5	0.12	0.23	0.35	0.31	0.01	0.03	0.001	10.20

8-Seed weight per pod (g)

The concentration (0.1% v/v) exerted a favorable influence on this trait (0.23 g) in comparison with the control (0.22 g), and several superior plants were selected. The coefficient of variation (CV %) exhibited a range from moderate to high across the concentrations, with the highest value observed at the concentration of 0.3% v/v (42.69%). The range was found to be wider at the concentrations (0.1% v/v and 0.3%) (0.06 g) in comparison to the control (0.04 g), indicating genetic variation in this trait due to the effects of different concentrations of the mutagen.

Table (11) Statistical indicators of the effect of the EMS mutagen on the seed weight trait per pod (g) of Sb239 (M2) soybean plants

Mutagen concentration V/V %	Number of selected plants	range	Minimum value	Supreme Value	Average quality	Standard error	standard deviation	Contrast	Coefficient of variation
0	11	0.04	0.19	0.23	0.22	0.01	0.05	0.003	24.89
0.1	27	0.06	0.19	0.26	0.23	0.01	0.08	0.007	37.52
0.2	24	0.05	0.16	0.21	0.19	0.01	0.07	0.006	41.16
0.3	13	0.06	0.12	0.18	0.16	0.01	0.06	0.004	42.69
0.4	5	0.05	0.16	0.21	0.19	0.01	0.03	0.001	16.64

9-Weight of 100 seeds:

Table (12) shows the positive effect of the chemical stimulant EMS on the weight of 100 seeds, which was most pronounced at a concentration of 0.1% v/v (17.27 g), where it had a positive and stimulating effect and many superior plants were selected compared to the control (17.19 g). The coefficient of variation (CV%) was low at the concentrations studied compared to the control, and was highest at a concentration of 0.1% v/v (16.94%), The range was wider at most of the concentrations studied, and it was highest at the 0.1% v/v concentration, reaching 4.78 g compared to the control (4.12 g). This indicates the role of the chemical mutagen EMS in creating genetic variation, particularly for this trait, influenced by the different concentrations of the mutagen. Our results are fully consistent with the studies [18], [15].

Regarding the increase in the weight of 100 seeds in plants treated with low and medium doses.

Table (12) Statistical indicators of the effect of the EMS mutagen on the 100 seed weight (g) trait of Sb239 (M2) soybean plants

Mutagen concentration V/V %	Number of selected plants	range	Minimum value	Supreme Value	Average quality	Standard error	standard deviation	Contrast	Coefficient of variation
0	11	4.12	15.11	19.23	17.19	1.42	4.72	22.30	27.47
0.1	27	4.78	15.44	20.22	17.27	0.56	2.92	8.57	16.94
0.2	24	3.94	14.38	18.33	16.36	0.53	2.63	6.94	16.10
0.3	13	4.50	12.49	16.99	16.00	0.63	2.28	5.24	14.31
0.4	5	2.67	6.66	9.33	8.65	0.37	0.83	0.69	9.60

10-The number of pods in a plant:

The EM concentrations (0.1% v/v) and (0.2% v/v) yielded higher values than the control. In particular, the mean concentration of 0.1% (v/v) (147.36 pods) was found to be significantly higher than the control (142.08 pods), which exhibited a positive and stimulating effect. Furthermore, some superior plants were selected (see Table 13), with some reaching up to 107.00 pods. The coefficient of variation (CV%) attained its maximum value at the (0.2% v/v) concentration (4.80%) in comparison to the control, and the range was most extensive at the (0.3% v/v) concentration (38.33 ears) in comparison to the control (20.00 pods), suggesting genetic variation in this trait due to the varying concentrations of the studied mutagen and, consequently, an augmented importance of selection for this trait in subsequent generations. It was demonstrated that low concentrations were associated with a decline in the number of pods and seeds, while the number of pods and seeds per plant exhibited an increase at relatively high concentrations. The findings of this study did not align with those of [22], as no correlation was observed between the number of pods and the concentration of EMS. The findings pertaining to the quantity of pods per plant were also incongruent with those of the reference sample. The number of pods exhibited an increase at low concentrations and subsequently decreased as the concentration increased.

Table (13) Statistical indicators of the effect of the EMS mutagen on the trait of the number of pods in the plant for soybean plants Sb239 (M2)

Mutagen concentration V/V %	Number of selected plants	range	Minimum value	Supreme Value	Average quality	Standard error	standard deviation	Contrast	Coefficient of variation
0	35	28.50	92.00	120.50	111.60	0.67	2.62	6.88	1.32
0.1	25	22.00	139.67	161.60	150.30	1.35	4.87	23.74	2.67
0.2	24	23.33	126.67	150.00	139.33	2.07	6.55	42.90	4.35
0.3	17	20.617	81.75	102.36	114.40	2.77	8.32	69.00	6.14
0.4	6	8.00	41.50	49.50	45.83	4.00	5.65	32.00	10.10

11- Weight of pods per plant (g):

As illustrated in Table (14), the chemical growth regulator EMS (56.85 g) at a concentration of 0.1% v/v had a favourable effect on pod weight, particularly in comparison to the control (53.62 g). This observation indicates that the application of EMS led to the selection of numerous superior plants, suggesting a positive and stimulating effect on plant development. The coefficient of variation (CV%) was moderate in the control group, At the same time, it ranged from low to high at the concentrations, with the highest value recorded at the 0.1% v/v concentration (24.17%). The range was wider at many of the concentrations studied, with the highest range observed at the 0.2% v/v concentration, where it reached 19.93 g compared to the control (7.15 g). This demonstrates the role of the chemical mutagen EMS in increasing the genetic variation of this trait, paving the way for selection and breeding to increase pod weight in the plant. The quantity of seeds per pod is also documented.

Concerning the findings concerning the number of seeds per pod, these did not correspond with the results reported by [4], who stated that the number of pods ranged from 20.4 to 30.0 pods/plant. However, the results for the number of seeds per pod were consistent with those of the aforementioned study, attaining 2.2 seeds/pod.

Table (14) Statistical indicators of the effect of the EMS mutagen on the trait of pod weight in the plant (g) for Sb239 (M2) soybean plants

Mutagen concentration V/V %	Number of selected plants	range	Minimum value	Supreme Value	Average quality	Standard error	standard deviation	Contrast	Coefficient of variation
0	11	7.15	49.50	56.65	53.62	2.41	8.00	64.11	14.93
0.1	27	12.61	54.20	66.81	56.85	2.64	13.74	188.97	24.17
0.2	24	19.93	49.00	68.93	49.31	2.29	11.22	125.97	22.75
0.3	13	10.50	34.10	44.60	37.13	2.40	8.67	75.22	23.35
0.4	5	6.50	5.25	32.00	28.07	0.79	1.77	3.14	6.31

12-Weight of seeds per plant/g

The weight of seeds in plants (i.e. plant productivity) is one of the most significant traits that are the focus of study, and the primary objective of those engaged in plant breeding. This trait is intricate in nature due to its susceptibility to the interplay of numerous genes, thereby complicating the identification of the specific genes responsible for augmented yield. As illustrated in Table (15), the chemical mutagen EMS (0.1% v/v) exerted a favourable influence on seed weight, with the optimal outcome observed at this concentration (34.75 g). This concentration elicited a positive and stimulatory effect, resulting in the selection of numerous superior plants, as compared to the control (32.31 g). The coefficient of variation (CV%) exhibited a range from moderate to high at the concentrations that were the subject of this study, with the highest value being recorded at a concentration of 0.3% v/v (40.18%). The highest range was observed at a concentration of 0.2% v/v, reaching 6.65 g, while the control value was 7.50 g. This indicates the effect of the EMS mutagen on this trait and paves the way for the selection of plant productivity traits.

Table (15) Statistical indicators of the effect of the EMS mutagen on the seed weight trait in the plant (g) of Sb239 (M2) soybean plants

Mutagen concentration V/V %	Number of selected plants	range	Minimum value	Supreme Value	Average quality	Standard error	standard deviation	Contrast	Coefficient of variation
0	11	7.5	28.00	35.50	32.31	2.47	8.20	67.30	25.39
0.1	27	6.35	31.75	38.10	34.75	2.57	13.35	178.43	38.44
0.2	24	6.65	24.65	31.30	27.98	2.26	11.11	123.59	39.73
0.3	13	6.03	15.93	21.96	19.74	2.20	7.93	62.96	40.18
0.4	5	5.25	9.00	14.25	12.02	0.95	2.13	4.54	17.72

13-Protein percentage:

As demonstrated in Table 16, the chemical modifier EMS exhibited no substantial positive impact on protein content, with the most notable discrepancy observed at a concentration of 0.1% v/v (29.74%) in comparison to the control (27.05%). The coefficient of variation (CV %) exhibited moderate levels in the control group. However, it demonstrated a range from low to high at the concentrations that were examined. The highest recorded value was observed at a concentration of 0.3% v/v (12.35%), and the range was found to be more extensive at numerous seed concentrations. The widest range was recorded at a concentration of 0.3% v/v, reaching (9.75%) compared to the control (10.05%). This finding suggests the impact of the EMS mutagen on this particular trait and paves the way for the selection of protein content. Research has also indicated that EMS is superior to other chemical and physical mutagens when used at low concentrations, particularly for modifying the chemical composition of soybeans to increase protein and oil content [19].

Table (16) Statistical indicators of the effect of the EMS inhibitor on the protein trait (%) of Sb239 (M2) soybean plants

Mutagen concentration V/V %	Number of selected plants	range	Minimum value	Supreme Value	Average quality	Standard error	standard deviation	Contrast	Coefficient of variation
0	11	10.05	21.92	31.97	27.05	1.05	3.45	11.90	11.70
0.1	27	6.70	24.66	31.36	29.74	0.49	2.52	6.35	8.06
0.2	24	8.22	20.40	28.62	26.06	0.75	3.24	10.49	11.68
0.3	13	9.75	19.78	29.53	24.47	0.93	3.25	10.56	12.35
0.4	5	6.39	18.27	24.66	25.43	1.28	2.78	7.72	9.65

Conclusions and Recommendations:**Conclusions:**

-The EMS mutagen has been demonstrated to be efficacious in inducing novel mutations in numerous economic traits.

-The most effective concentration was determined to be 0.1% v/v, with a gradual decline in values observed as treatment concentrations increased (0.2, 0.3, and 0.4%) v/v, particularly in terms of yield, yield components, and protein content in the selected Mutant plants.

Recommendations:

-Continue breeding work on plants selected over many generations to develop new genetic forms with good yield and quality characteristics.

The work should be continued using the EMS mutagen on other soybean genotypes and at different concentrations

References:

- [1]-H. Adlercreutz and W. Mazur, Phyto-estrogens and western diseases. *Ann. Med.* 29:95-120. (1997)
- [2]-A. Al-Adhari and A. H. Muhammad, Fundamentals of Genetic. Directorate of Dar AL-Kutub for printing and publishing. University of Mosul- Iraq. (1987)
- [3]-A. JrLH, L. Zhang, K.J. Boote and B.A. Hauser, Elevated temperature intensity, timing, and duration of exposure affect soybean internode elongation, mainstem node number, and pod number per plant. *The Crop Journal*. 6:148-161. (2018)
- [4]-A. Al-Sahouki and M. Majeed, Soybeans: Production and Improvement. Dar Al-Hikma for printing and publishing, College of Agriculture, University of Baghdad. (1991)
- [5]-W. An, H. Zhao, Y. Shandong, Y. Wang, Q. Li, B. Zhuang, L. Cong, and B. Liu , Genetic diversity in annual wild soybean (*Glycine soja* sieb. ET zucc.) And cultivated soybean (*G. max*. Merr.) From different latitudes in china. *Pak. J. Bot.*, 41(5): 2229-2242. (2019)
- [6]-A.O.A.C, Association of Official Analytical Chemists Official methods of analyses, Virginia. (1990)
- [7]-L. Chen, L. Duan, M. Sun, Z. Yang, H. Li, H. Hu, H. Yang and L. Liu , Current trends and insights on EMS mutagenesis application to studies on plant abiotic stress tolerance and development. *Front Plant Sci*. 13:1052569. (2023)
- [8]-Q. Cheng, L. Dong, T. Su, T. Li, Z. Gan and H. Nan, CRISPR/Cas9-mediated targeted mutagenesis of *GmLHY* genes alters plant height and internode length in soybean. *BMC Plant Biol*. 19:562. (2019)
- [9]-M. De Méo, M. Laget and M. Castegnaro, Evaluation of methods for destruction of some alkylating agents. *American Industrial Hygiene Association Journal*. 51(9):505- 509. (1990)
- [10]-P. Gopinath and P. Pavadai, Morphology and Yield parameters and Biochemical analysis of Soybean (*Glycine max* (L.) Mrr.) Using Gamma rays, EMS and DES treatment. *International Letters of Natural Sciences*. 35:50-58. (2015)
- [11]-H.L. Hanan, M. Abdalla and A.Farag, Radio-stimulation of phytohormones and bioactive components of coriander seedlings. *Turkish J. Biochem*. 36, 230–236. (2011)
- [12]-I. Jonckheere, S. Fleck, K. Nackaerts, B. Muysa, P. Coppin, M. Weiss and F. Baret, Review of methods for in situ leaf area index determination Part I. Theories, sensors and hemispherical photography. *Agricultural and Forest Meteorology*. 121: 19–35. (2004)
- [13]-R. Karthika and B.S.Lakshmi, Effect of Gamma Rays EMS on two varieties of Soybean. *Asian Journal of plant sciences* 5(4):721-724. (2006)
- [14]-M.H. Khan and S.D. Tyagi, Studies on induction of chlorophyll mutations in soybean, *Glycine max* (L.) Merrill. *Frontiers of Agriculture in China*.3 (3):253-258. (2009) <https://doi.org/10.1007/s11703-009-0058-2>
- [15]-V.A. Kumar, N. Vairam and R. Amutha Effect of physical mutagen on expression of characters in arid legume pulse cowpea (*Vigna unguiculata* (L.) Walp.). *Electr. J. Plant Breed*. 1, 908–914. (2010)
- [16]-R.A. Laskar and S. Khan, Assessment on induced genetic variability and divergence in the mutagenized lentil populations of macrosperma and macrosperma cultivars developed using physical and chemical mutagenesis. *PLoS* 1 12:e0184598. (2017)
- [17]-A. Micke and B. Donini, Use of induced Mutation in Improvement of seed prorogated Crops. Joint FAO Vienna. Austria pp: 2-10. (1981)
- [18]-P.G.C. Odeigah, A.O. Osanyinpeju and B.O. Myers. Induced mutations in cowpea (*Vigna unguiculata* L.Walp) (Leguminous) Vienna, Austria. (1998)
- [19]-P. Pavadai, M. Girija and D. Dhanavel, Effect of Gamma Rays, EMS, DES and COH on protein and oil content in Soybean. *Journal of Eco biotechnology*. 2(4):47-50. (2010)

- [20]-S. Plesnik, The evolution of some quantitative traits in M1, generation in Soybean after laser emission and ethylene mine treatment. *Acta Faculties Reerum Natralium Universities Comenianae. Genetic ET Biologia Molecular* is 24-25:105-113. (1993)
- [21]- M. Romero, R.Ortízy and M.Ponce, Radio sensibilidad de semillas de soya a los rayds gamma CO60. *Cultivos tropicales*21 (2): 43-47. Instituto Nacional de Ciencias Agricola's (INCA). Cuba. (2000)
- [22]-Z. Sagle, M.I. Tutluer, H. Peskircioglu, Y. Kantoglu and B. Kunter , Determination of effect of chemical mutagen EMS on TAEK A-3 and TAEK C-10 mutant soybean varieties in M1 generation. *EKIN Journal of Crop Breeding and Genetics*. 3(1):19-24. (2017)
- [23]-A. Shamshad, M. Rashid, L. Jankuloski, K. Ashraf, K. Sultan, S. Alamri, M.H. Siddiqui, T. Munir and Q. Zaman, Effect of ethyl methane sulphonate mediated mutation for enhancing morpho-physio-biochemical and yield contributing traits of fragrant rice. *Peer.11:e15821*. (2023)
- [24] –A. Tambe, and B. Apparao, Gamma Rays induced mutation in Soybean for yield-contributing traits. *Induced plant mutation in the genomics. Era. FAO, Rome*. 95-96. (2009)
- [25]-E. Wójcik-Gront, D. Gozdowski, A. Derejko and R. Pudelko , Analysis of the impact of environmental and agronomic variables on agronomic parameters in soybean cultivation based on long-term data. *Plants*. 11:2922. (2022)
- [26]-J. Yuan, N. Bellaloui, N. Lakhssasi, S.M. Abu Bakr, S. Kassem, Z. Kassem, S. Kassem, C. Barnes, A. McLelland, B. Brown , W. Adams, T. El-Mellouki, K. Meksem and M.A. Kassem , Evaluation of yield performance of Soybean mutant FM6-847 in North Carolina. *Atlas Journal of Plant Biology*. 96-105. (2020)
- [27]-J. Zhang and O. Yu, Metabolic engineering of is flavone biosynthesis in seeds. In *Modification of seed composition to promote health and nutrition*. (2009)
- [28]-Z. Zhou, N. Lakhssassi, M.A. Cullen, A. El Baz, T.D. Vuong, H.T. Nguyen, K. Meksem , Assessment of phenotypic variations and correlation among seed composition traits in mutagenized soybean populations. *Genes*. 10:975. (2019).
- [29]-S. Durre, Mutagenesis-based plant breeding approaches and genome engineering: A review focused on tomato. *Mutation Research-Reviews in Mutation Research*.(2023).

