Study of seed mutation by EMS mutant and planting density in growth and yield and quality of Broad bean

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Abstract

The field experiment was carried out during the winter season of 2025-2026 in the field of the Department of Field Crops at the College of Agriculture - Al-Qasim Green University. The aim of study to determine the effect of mutation by EMS mutant and plant density on growth and yield and quality of Broad bean . The experiment was included two factors: The first factor was the optimal mutagenic levels in the laboratory were (M0: control treatment, water only), (M1 : treatment exposed to 0.25% mutagenic substance), (M2: treatment exposed to 0.50% mutagenic substance). The second factor was the planting density (D1 : planting density 25×50 cm), (D2:20×50 cm), (D3: 25×30 cm), (D4: 20×30 cm). The experiment was conducted by using a randomized complete block design (RCBD) with a factorial experiment arrangement with three replicates. The results were showed : EMS concentration levels had a significant effect, with concentration (M2) was given increase in the number of main branches, number of pods per plant, seed 100 weight, plant yield, and protein percentage. Concentration (M1) was superior in total leaf area. D3 treatment was superior in the number of main branches, number of pods per plant, seed 100 weight, plant yield, and protein percentage. While D2 treatment was superior in total leaf area. Interaction treatments had a significant effect on most traits, the interaction (D3 x M2) was resulted in the highest average for the number of main branches, number of pods per plant, seed 1 00 weight, plant yield, and protein percentage. The interaction (D2 x M1) was superior in the total leaf area.

Introduction

Broad beans are annual herbaceous plants belonging to the legume family (Fabaceae). They are winter legumes which growing best during cool and temperate periods.

Broad beans (Vicia faba L.) are a widely cultivated legume crop worldwide due to their high nutritional value. It is grown in many countries for their green pods or dried seeds, which serve as a staple food for humans and animal feed, it contributes significantly to a balanced diet because of containing from high amount of plant protein, carbohydrates, vitamins, fats, and cellulose and it also plays a valuable environmental role in improving soil fertility through nitrogen fixation by the bacterial nodules in their roots. In addition to their nutritional and economic value, the protein extracted from broad beans can be used industrially due to its ability to form a gel, these properties depend on

ISSN 2072-3857

specific conditions such as pH and temperature[1]. It can also be used in the production of foams, emulsions, and adhesives. Furthermore, broad bean husks are of great importance in the animal feed industry. China is now among the world's largest producers, accounting to approximately 60% of total production [2].

Mutation is one of the most prominent and targeted methods in plant breeding to increase production and improve quality. Artificial mutagens allow for genetic modification without affecting the characteristics of the original variety, and they make an effective tool, especially in stressful environments. Therefore, mutation has considered one of the best approved methods for improving crop productivity. It is also used for producing of plants with traditionally undesirable characteristics in response to the increasing which need new genetic variations that achieve high-efficiency varieties, greater resilience, and higher nutritional value [5]. Mutation defines as a change that occurs in the sequence or number of nitrogenous bases in DNA, leading to the formation of new nucleotide sequences whose effects are inherited by subsequent generations through phenotypic traits. Mutations often result from a difference in the number of chromosomes or changes in the structure of the chromosome. These changes may occur naturally and spontaneously or induced by using of mutagens such as radiation or chemicals. If a mutation occurs at the gene level, it may lead to a change in its structure or activity, which in turn affects the vital functions, such as the production of enzymes or hormones. The mutation may disable the gene, reduce efficiency, or increase efficiency in producing proteins or carrying out various vital activities [6]. Many chemicals are capable of inducing genetic mutations or changes in genes. Recent studies have shown that dozens of these substances can cause genetic changes in cells, tissues, or organisms at specific concentrations and for a certain period of time. Among these chemicals are Ethyl Methane Sulfonate (EMS), Diaethyl Sulfate, and Colchicine. These substances exhibit effects similar to those resulting from radiation exposure. These mutagens cause changes in the sequence of DNA bases, leading to the emergence of new genetic traits that can be used to improve crops. Planting density play an important role in determining plant growth and branching, so the choose of the appropriate density between plants reduces shading and competition for resources, and it allows to plants grow more freely and form more branches, thus it increases the number of flowers and pods and enhancing the overall crop productivity. Conversely, narrow density lead to mutual shading and competition for light and nutrients, so it reduces branching growth and limits plant productivity [3]. Therefore, management of planting density appropriately is a key factor for improving growth, branching, and the final yield of broad beans. These branches help the plant increase the number of leaves and leaf area, which in turn helps the plant absorb light and photosynthesize. Branching also helps the plant distribute food between the leaves and branches, and between the flowers and pods. Branching has affected by both genetics and environment. Important factors include light, leaf area exposed to light, and nutrients in the soil. The plants grow in perfect conditions

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produce more branches, high pod yield. Branching also helps the plant adapt to different conditions [4]. So this study was conducted for Determining of the growth and yield which affected by mutation seed and planting density between rows and between the optimal plants.

Materials and Methods

The experiment was conducted during the 2025-2026 winter season at the field of the Field Crops Department, College of Agriculture, Al-Qasim Green University. The experimental field was plowed perpendicularly using a moldboard plow, leveled, and divided according to a randomized complete lock design(RCBD) in factorial experiment with three replications. Five soil samples were randomly taken from different locations within the experimental field before planting to a depth of 0-30 cm (Table 1). Seed planting was carried out in rows in experimental units in the experimental field, Planting density between rows and plants varied according to the experimental treatments. The field was divided according to the factorial experiments arrangement into RCBD design with three replications, and the area of each experimental unit was (2.5 x 2.5) m2, with a 1 m density between units. The experiment included two factors: The first factor: Optimal mutagenicity levels in the laboratory experiment, which were: M0: Control treatment (water soaking only).

M1 : Exposure treatment (0.25% mutagenic agent).

M2: Exposure treatment (0.5% mutagenic agent).

The second factor: Planting density

D1 : Planting density (50 x 25) cm (recommendation: Lishan et al., 2022)

D2: Planting density (50 x 20) cm

D3: Planting density (30 x 25) cm

D4: Planting density (30 x 20) cm

Seed planting was taken on November 7, 2025, with two seeds per hole and an average of 24 plants per experimental unit. Irrigation was administered at germination, and fertilizer was added according to the recommendation (Table 2).Treatments were randomly distributed, and the experimental units were marked. The experiment was subsequently irrigated based on soil moisture. Thinning was carried out, leaving one plant per hole, seven days after emergence. Weeding and hoeing were performed to remove any accompanying weeds.

Table 1 . Physical and chemical properties of the field experiment soil

Property	Values
Electrical Conductivity (EC) ($dS \cdot m^{-1}$)	3. 6
Soil pH	7. 3
Available Nitrogen (mg/kg^{-1})	1 . 21

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Available Phosphorus (mg/kg^{-1})	24.2
Available Potassium (mg/kg^{-1})	381
% of Organic Matter	1 . 36
Sand (g/kg^{-1})	224
Clay (g/kg^{-1})	345
Soil Texture	Clay loam, silt

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Table 2. Fertilizer recommendation for the field experiment

Fertilizer name	Quantity in experimental units	Recommendation
Superphosphate	40g	P2O5 at a rate of 100 kg/hectare in a single- application Before planting.
Urea	8g	40 46%)) kg/N per hectare in two applications: the first one month after planting and the second one month after the first application.

The study traits were total leaf area, number of main branches, number of pods per plant, seed 1 00 weight, plant yield, and protein percentage.

Statistical Analysis

The results were analyzed by using the GenStat statistical analysis program according to a randomized complete block design (RCBD) with a factorial experiment arrangement and three replications. The arithmetic means were compared based on the least significant difference (LSD) at a probability level of 0.05 .

Results and Discussion

1.Total Leaf Area ($cm^2/plant^{-1}$)

The results in Figure (1 .1) appeared a significant effect between the concentrations of the EMS mutagen. M1 treatment gave the highest average total leaf area 1238.9 cm^2/plant-1 , compared to the control treatment (M0), which gave low average 1101. 9 cm^2/plant-1 . This is attributed to the fact that EMS mutagen stimulated enzyme activity and activated genes responsible for cell division and increased leaf size in the plant[7]. The results in Figure (1.2) showed significant differences when using different planting density. D2 treatment gave high average of total leaf area trait 1281.7 $cm^2/plant^{-1}$, compared to D1 which recorded the lowest average 1080.8 $cm^2/plant^{-1}$. This is attributed to the fact that it provided a suitable distance between rows, reducing competition for light, water, and nutrients, while simultaneously maintaining perfect plant density. This helped the leaves to expand better, and increased leaf area in this treatment. These results are consistent with those found [8]. The results in Figure (1. 3) indicate an interaction between mutagen levels and planting density. The interaction treatment (D2 x M1) gave high average of total leaf area 1760.8 cm^2/plant-1 , compared to the interaction treatment (D2 x M0), which gave low average 661 .8 cm^2/plant-1 .

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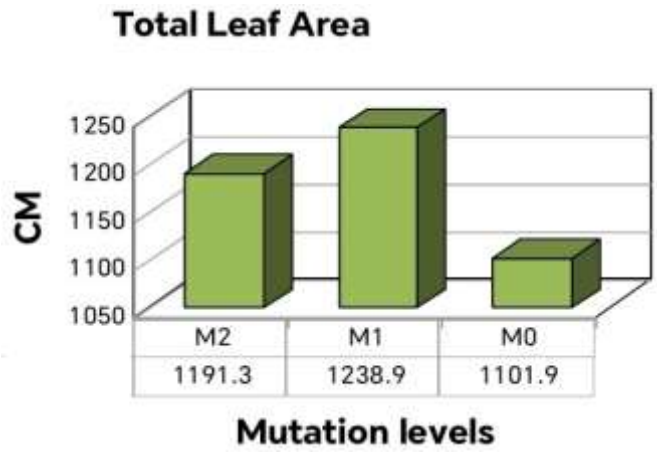


Figure (1.1.) Effect of mutation levels on total leaf area under the lowest significant difference value: 11.59

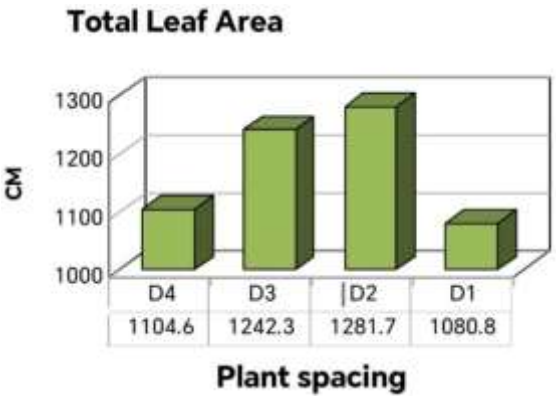
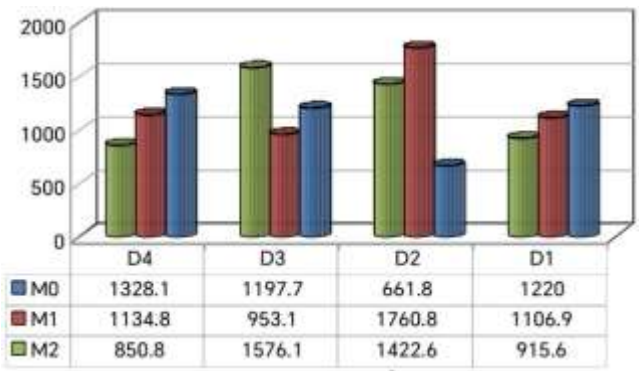


Figure (1 .2 .) Effect of planting density on total leaf area under Least Significant Difference value: 13.38 .



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Figure (1.3 .) Interaction between mutation levels and planting density under the least significant difference value: 23.17

2.Number of Main Branches (branch. plant-1)

The results in Figure (2.1.) noted a significant effect between the concentrations of the chemical mutagen. M2 treatment gave the high average in number of main branches 12.913 branch . plant-1 , compared to the control treatment (M0), which gave low average 8.816 branch . plant-1 . This is due to the plant's reaction to the EMS mutagen, which stimulates cell activity and increases the plant's ability to branch. This trait is greatly affected by external factors, as the mutagen increases the formation of branching buds in response to the activator and vegetative growth promoter (mutagene), which is involved in many physiological mechanisms within the plant [9،10]. The results in Figure (2.2.) showed significant differences by using different planting density. D3 treatment gave high average in number of main branches 1 3.251 branch . plant-1 , while D4 treatment recorded low average 9,089 branch . plant-1 . This may be attributed to the positive effect of the close spacing between rows, which played a significant role in stimulating lateral buds to form branches within the optimal planting distance between plants. Conversely, shorter distances had a negative impact due to increased crowding between plants, while larger distances did not exhibit the competition that stimulates plants to produce the maximum number of branches. These results are consistent with those found [11]. The results in Figure (2.3) regarded the interaction between the levels of EMS chemical mutation and planting density indicated a significant difference. The interaction (D3x M2) gave high average in number of main branches 15.753 branch . plant-1 , compared to the interaction (D4x M0), which gave low average 7.867 branch . plant-1 .

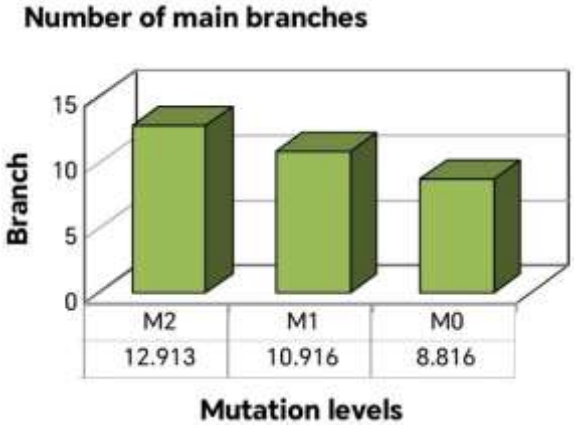
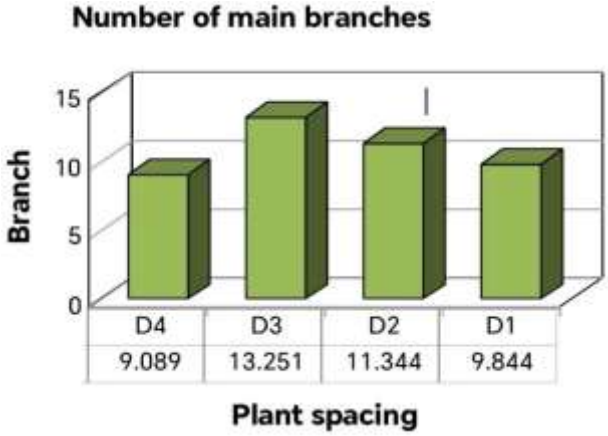


Figure (2.1.) mutation levels on the number of main branches (plant . branch-1) under the lowest significant difference value: 0.267

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Figure (2 .2.) effect of planting distances on the number of main branches (branch . plant-



1) under the lowest significant difference value: 0.3090

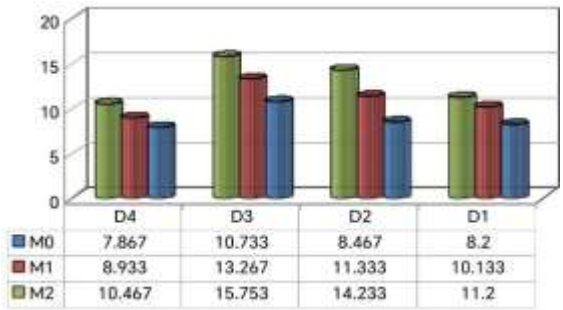


Figure (2.3.) the interaction between planting density and mutation levels under the least significant difference value: 0.5352

3.Number of pods per plant (pod . plant-1)

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The results in Figure (3.1.) showed significant differences exist between concentrations of the chemical mutagen EMS. M2 treatment gave high average in a number of pods per plant 17.467 pods . plant-1 , compared to the control treatment (M0), which gave low average 13.065 pods . plant-1 . The reason for the increase in the number of pods per plant in the mutagen treatment is attributed to the increase in the number of branches, in addition to the increase in other vegetative traits, which positively affected the number of pods formed in the plant. This substance works to activate plant growth by stimulating cell division and improving hormonal balance within the plant, which led to an increase in the formation of flower buds and thus an increase in the number of pods [12].

The results in Figure (3.2,) registered significant differences by using different planting density. D3 treatment gave high average in number of pods per plant 17.463 pods . plant-1 , while D4 treatment recorded low average 12.273 pods . plant-1 . The superiority of this treatment was attributed to the fact that it provided a suitable plant density, which contributed for stimulating the formation of flower buds, improving growth conditions, and reducing competition between plants for light and nutrients. This was reflected in the overall growth efficiency of the plant and an increase in the number of pods formed per plant. This is also attributed to the superiority of this treatment in the number of main branches, which led to an increase in flowering sites and consequently an increase in the number of pods per plant [13 ،14].

The results in Figure (3.3.) indicated that the interaction between the levels of mutagenicity of the EMS chemical and the planting density was significantly affected, the interaction (D2 x M2) gave high average in a number of pods per plant 1 9.633 pods . plant -1 , compared to the interaction (D4 x M0), which gave low average 1 0.21 5 pods . plant-1 .

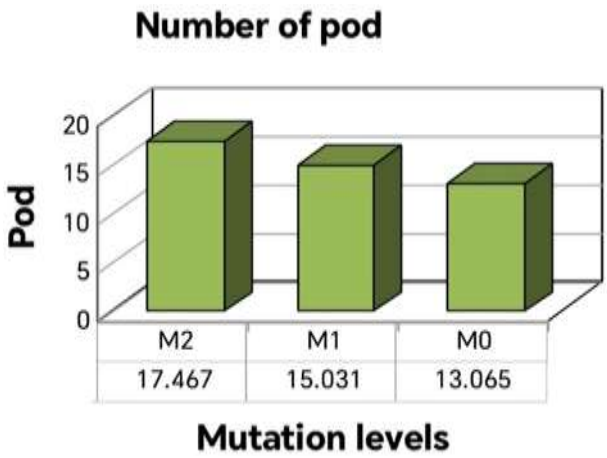


Figure (3.1.) mutation levels a number of pods per plant (pod . plant-1) under the lowest significant difference value: 0.2363

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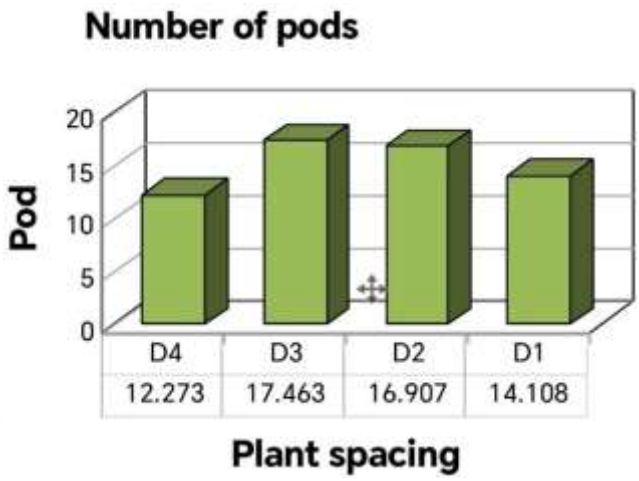


Figure (3 .2.) effect of planting density in a number of pods per plant (pod . plant-1) under the least significant difference value: 0.2728

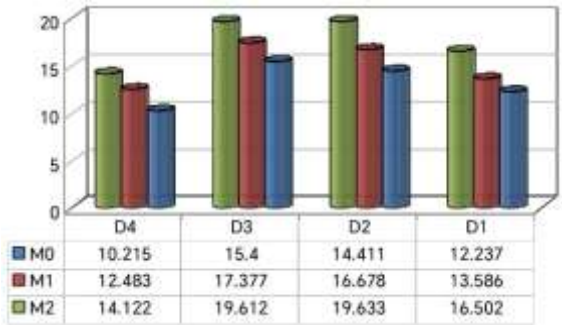


Figure (3.3.) Interaction between planting density and mutation levels under the least significant difference value: 0.4725

4.Seed 100 weight (g)

The results in Figure (4.1.) appeared a significant effect between concentrations of the chemical mutagen EMS. M2 treatment resulted in high average in seed 1 00 weight 152.99 g, compared to the control treatment (M0), which resulted in low average seed 100 weight 126.14 g. This increased came from the mutagen concentration which increased there number of branches than increase of the number of leaves in the plant, thus increasing photosynthesis, dry matter accumulation, and compound accumulation in the seeds. These results are consistent with those found [15 ،16].

Figure (4.2.) indicated significant differences in different planting distances. D3 treatment gave high average in seed 100 weight 153.69 g, while D4 treatment recorded low average 122.86g. The superiority of this treatment was attributed for achieving an optimal plant density, which led to an improved balance between vegetative growth and the formation of fruiting parts. This was reflected in the increased number of main and secondary branches, the number of pods per plant, and the number of leaves, which led to an increase in photosynthetic efficiency per unit area. This improvement led to an increase in the production and transport of photosynthetic products during the seed filling stage, providing better nutrition for the seeds inside the

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pods and contributing to an increase in their filling degree and, consequently, a higher seed weight. In contrast, wider distances reduced the efficiency of utilizing the area per unit area, while very narrow distances led to increased shading and competition between plants, which reduced filling efficiency and seed weight. This is consistent with As indicated [17،18].

Figure(4.3.)registered the interaction between the levels of EMS mutagen and planting density. This interaction was significantly affected in the interaction (D3 x M2) resulted in high average seed 100 weight 167.23g compared to the interference (D4 x M0) which gave low average 111.2 g.

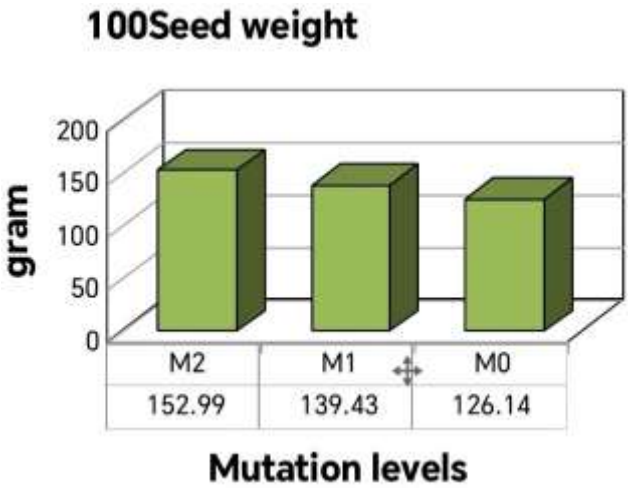


Figure (4.1.) mutation levels in seed 100 weight (g)under the Least Significant Difference value: 0.757

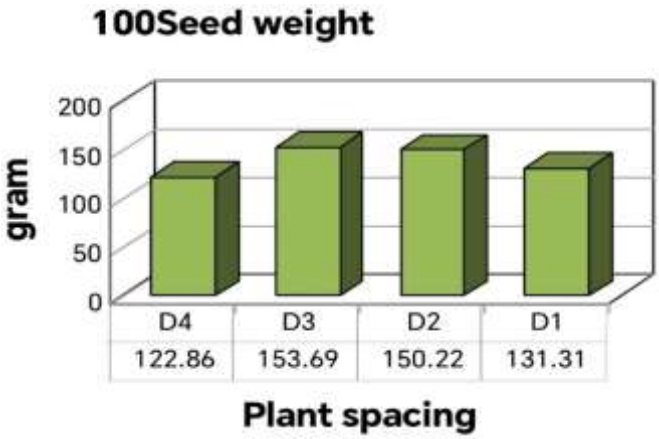


Figure (4 .2.) Effect of planting density in seeds 1 00 weight (g) under the Least Significant Difference value: 0.874

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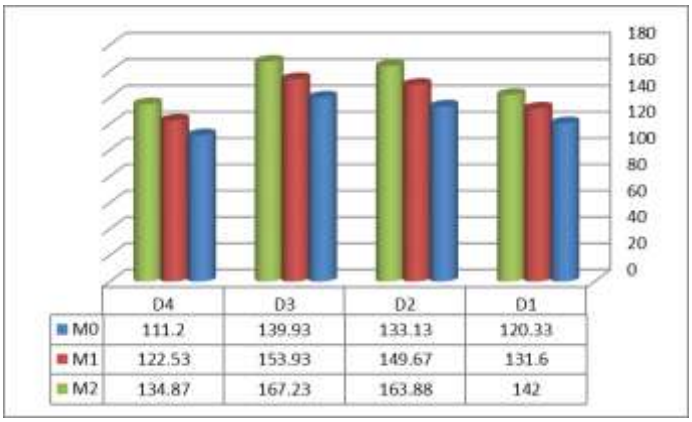
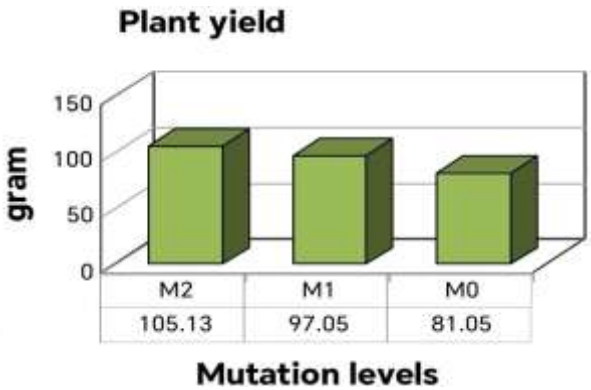


Figure (4 .3.) Interaction between planting density and mutation levels under the least significant difference value: 1.514

5.Plant Yield (g . plant-1)

The results in Figure (5 .1) showed a significant effect between the concentrations of the chemical mutagen EMS. M2 treatment gave high average in plant yield 105.13 g . plant-1 , compared to the control treatment (M0), which gave low average 81 .05 g . plant^{-1} . The increased in the yield was come to the mutagenic agent stimulating hormonal balance within the plant, leading to an increase in yield components. High concentrations of the mutagenic agent resulted in an increase in the number of pods per plant, the number of seeds per pod, and the weight of 100 seeds [9]. The results in Figure (5.2) indicated significant differences by using different planting density. D3 treatment gave high average in plant yield 97.1 5 g . plant-1 , while D4 treatment recorded low average 88.73 g . Plant-1 . The superiority of this treatment was from the ideal balance it provided between plant density and the space available per plant. It granted the plant sufficient room to grow without intense competition. This balance led to improved light interception efficiency, increased photosynthetic efficiency, enhanced water and nutrient absorption. This improved yield-related traits such as the number of pods per plant, and the seed 100 weight. These results consistent with [19]. The results in Figure (5.3) indicated the interaction between the levels of mutagenicity of the EMS and planting density. This interaction was significantly affected, with the (D3 x M2) resulted in high average in plant yield 111 .83 g . plant-1 , while the (D4x M0) registered low average 77.87 g . plant-1 .



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Figure (5.1.) mutation levels in plant yield (g . plant-1) under the lowest significant difference value: 1.579

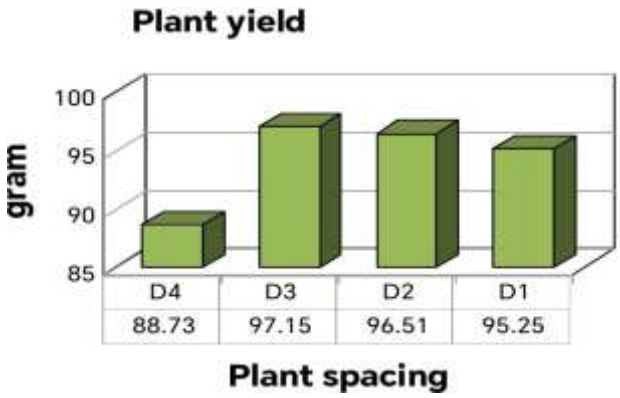


Figure (5 .2.) Effect of planting density in plant yield (g . plant-1) under the lowest significant difference value: 1.824

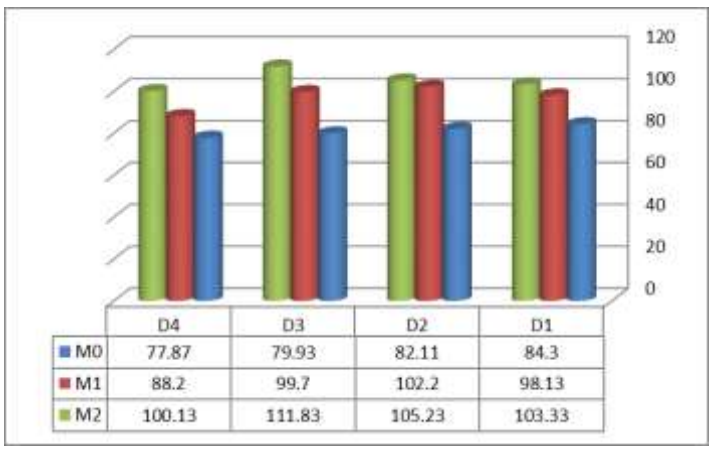


Figure (5.3.) Interaction between planting density and mutation levels under the least significant difference value: 3.159

6.Protein percentage (%)

The results in Figure (6 .1) showed a significant effect between the concentrations of the chemical mutagen EMS. M2 treatment gave high average in protein percentage 25.5%, compared to the control treatment M0, which gave lowe average19%. Regarding the protein percentage, this increase was attributed to the mutagenic effect in inducing genetic changes that stimulated the emergence of new genetic variations. This led to the activation of gene expression in some genes responsible for protein synthesis, resulted in increased protein accumulation in the seeds [20،21]. The results in Figure (6.2) showed significant differences by using different planting density. D3 treatment gave high average of protein percentage 24%, compared to D1 , which recorded low average 20.7%. The high percentage of protein in this treatment was from the moderate plant density,

ISSN 2072-3857

which provided suitable conditions that improved the efficiency of nitrogen fixation, uptake, and translocation to the seeds during the seed-filling stage. Additionally, the increased number of leaves helped to increase the efficiency of photosynthesis, as protein is directly related to the amount of nitrogen accumulated in the seeds. Therefore, improved nitrogen assimilation is reflected in increased protein content [22،23].The results in Figure(6.3) indicated the interaction between the levels of EMS chemical mutagenicity and planting density. This interaction was significantly affected, with the (D3x M2) yielded high average of protein percentage 29%, compared to the (D1 x M0), which yielded the low average in protein percentage of 1 8%.

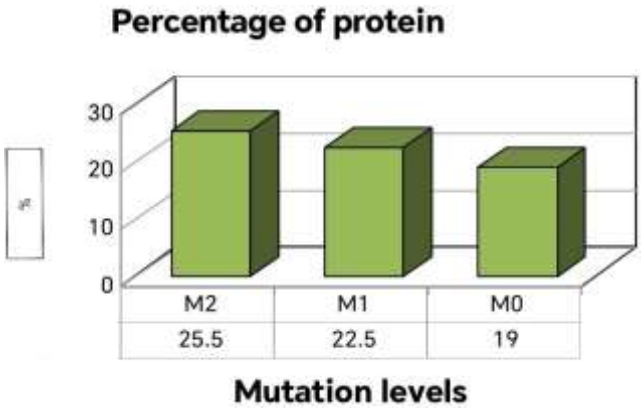


Figure (6.1.) mutation levels in the percentage of protein under the least significant difference value: 1.7

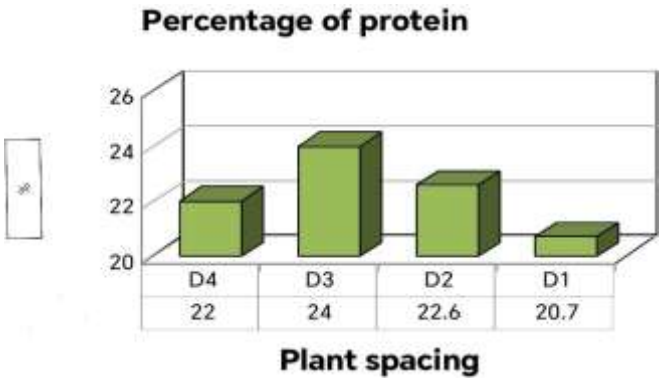
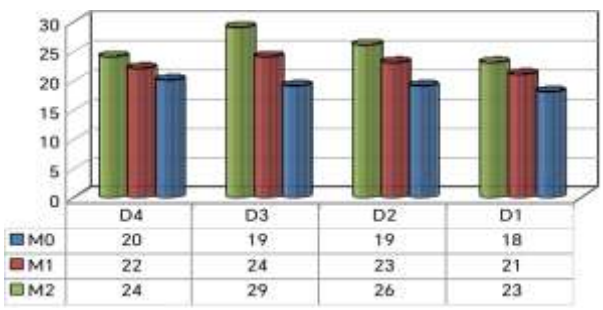


Figure (6 .2.) effect of planting density in the percentage of protein under the least significant difference value: 1.3



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Figure (6.3) the interaction between planting density and mutation levels under the least significant difference value: 2.6

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Conclusion

The 0.50% concentration of EMS exhibited superiority in enhancing most vegetative growth traits and yield components.

The treatment (D3), corresponding to the planting density of 25 × 30 cm, achieved the best performance for most vegetative growth traits and yield components.

The interaction treatment (D3 × M2) resulted in the best performance for most vegetative growth traits and yield components.

Recommendations

The use of a 0.50% concentration of the EMS mutagen is recommended due to its demonstrated positive effect on improving most vegetative traits and yield components. The 25 x 30 density is recommended due to its positive effect on improving most vegetative traits and yield components. The optimal interaction (D3 x M2) is recommended due to its demonstrated superiority in most vegetative and yield traits .

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