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Genetic Interaction Studies in Soybean Plants Prepared Using Sodium Azide and its Effect on Several Traits in the M2 Generation

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The research was done in the coastal region (of Syria) at Tishreen University during the agricultural seasons 2022-2023. The research included exposing the seeds of three genotypes of soybeans (sb44, sb239, and sb337) which were obtained from the General Authority for Agricultural research for mutagenesis using the chemical mutagen Sodium Azide (NaN3) in concentrations (0 mM, 1 mM, 2 mM, 3 mM, and 4 mM) with soaking it for different periods of time (4, 8, and 12 hours) within each concentration. In the purpose of studying the effect of Sodium azide concentrations and the periods of soaking on some morphologies, productive and quality traits of the three genotypes, in addition

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to determining the best concentration of sodium azide, soaking period and genotype of soybeans that has good productivity and quality. The results obtained indicated the presence of new mutations affected many morphological traits and production components, and the chemical mutagen is effective in causing mutations in soybeans with a different response from the genotype to the different concentrations and periods of soaking, as there was for the concentration C1 (1 mM) the biggest positive effect and stimulating on the most of the studied traits and dependent on it many plants were nominated and a significant decrease in most of the traits of the second generation (M2) plants with increasing the soaking period. Also a gradual decrease in the most of studied traits with the increasing concentrations of the mutagen and the increase of the soaking periods within each concentration. Additionally, there were the best interactions values at concentration C1 (1mM) and time T1 (4 hours of soaking).

Keywords: Soybean; chemical mutations; sodium azide; introduced mutations.

1. INTRODUCTION

Soybean *(Glycine max (L.) Merrill*) belongs to the *Fabaceae* family, also known as *Leguminosae*, under the genus *Glycine*, and the species *max [(L). Merrill*], hence its scientific name is *Glycine max (L.) Merr*. Cultivated soybean is a self-pollinating crop with a diploid chromosome number (2N=40) [1]. Soybean protein contains all essential amino acids, fatty acids, antioxidants, folic acid, and vitamin B, and it contributes to many therapeutic components [2]. Additionally, soybean consists of 40% protein, 35% carbohydrates, 20% oil, and 5% ash [3]. According to Maalla and Harba [4] soybean seeds contain 25-55% protein, 13-37% fat, and 20-32% carbohydrates. Rahman et al., [5] found that soybean seeds have high commercial value, containing approximately 40-45% protein, 20-22% oil, 20-26% carbohydrates, along with significant amounts of calcium and B complex vitamins.

Syria suffers from various economic problems in the agricultural sector, including securing vegetable oil and feed for the local market, leading tjo significant foreign currency expenditure to import these products, resulting in increased prices of oil, meats, eggs, and others. Therefore, there is a need to research advanced scientific methods to breed and improve crops to derive varieties with good agricultural specifications through traditional breeding methods [6] and improve agricultural processes, as well as adopt modern methods such as using mutagens, both physical and chemical [7-11].

Chemicals are an important means to induce mutations in plants, providing breeders with a wide range of genetic variations and leading to plant improvement in terms of quantity and quality [12]. Sodium azide (SA) is one of the most commonly used chemicals to induce mutations, causing gene mutations or point mutations (changes in one pair of bases or substitution with another pair of bases or duplication or deletion of one pair of bases). Borejko, [13] uses sodium azide to mutate many crops such as beans and soybeans, as demonstrated in inducing mutations in soybean plants using chemical mutagens. Oderigah et al., [14] studied two cultivars of Vigna unguiculata L. walp through treatment with NaN3 mutagen (0.1 Mm, 1 mM/2hr) and obtained morphological mutations leading to increased productivity using sodium azide (1Mm/24hr), where plant height reached 77 cm and 100-seed weight reached 19.8g compared to untreated plants with a height of 76 cm and 100-seed weight of 18.6g. Given the economic importance of soybean and related studies on improving its genetic traits, this research aims to: 1-Study the effect of various concentrations of the chemical mutagen sodium azide (NaN3) and soaking duration on some morphological, productive, and qualitative traits of soybean cultivars. 2-Determine the best concentration of the mutagen used, the optimal soaking duration, and the best genetic soybean cultivar with good productivity and quality.

2. METHODOLOGY

2.1 Plant Material and Source

Three genotypes of soybean were used in the research, obtained from the General Organization for Scientific Agricultural Research in Damascus, namely: (Sb44, Sb337, and Sb239).

2.2 Time and Location of Research Execution

The mutagenized seeds were planted in the first season on Friday, May 27, 222, and the seeds of

selected plants from the first generation were planted on Friday, May 26, 2023, in a private farm in the suburb of Damsarakhoo, located in the Latakia Governorate. Some readings were taken and protein analysis was conducted in the Scientific Research Laboratory at the Faculty of Agricultural Engineering - Tishreen University.

2.3 Parameters Studied

1-Mutagens: NaN3 (Sodium azide) chemical mutagen was used in the research. It was prepared as follows:

- C0 = 0 mM (control)
- C1 = 1 mM (dissolving 32.5 mg of sodium azide in 0.5 liters of water)
- C2 = 2 mM (dissolving 65 mg of sodium azide in 0.5 liters of water)
- C3 = 3 mM (dissolving 97.5 mg of sodium azide in 0.5 liters of water)
- C4 = 4 mM (dissolving 130 mg of sodium azide in 0.5 liters of water)

2-Soaking Duration: T0 = 0 hours (control), T1 = 4 hours, T2 = 8 hours, T3 = 12 hours.

3- Studied Genotypes: G1 = Sb44, G2 = Sb239, G3 = Sb337.

2.4 Soil Preparation for Cultivation

Deep plowing was conducted to loosen and aerate the soil, improve its physical properties, and eliminate weeds. Organic compost was added at a rate of 20 tons/hectare, along with potassium fertilizer at 120 kg/hectare (K2O) and phosphorus fertilizer at 152 kg/hectare (P2O5). The first batch of nitrogen fertilizer, urea (64%), was applied at a rate of 70 kg/hectare. The soil was then leveled well, experimental plots were established, and layout was done.

2.5 Seed Preparation for Cultivation in the First Season

Seeds of each genetic cultivar were divided into 13 samples, with 50 seeds per sample (i.e., 6500 seeds for each cultivar), and placed in plastic cups. All seed samples were soaked in plain water (50 seeds per treatment of the studied parameters) for 4 hours, based on recommendations by Ikhajiagbe et al., [15] indicating the optimal soaking duration for soybean seeds to increase the concentration of hereditary mutations (3-12 hours), followed by air drying for one hour. Subsequently, the samples were soaked in four different concentrations of the mutagen sodium azide (NaN3) (1, 2, 3, 4 mM) based on [16,17] for three different durations (4, 8, 12 hours), with the pH of the medium adjusted to 4 by adding a few drops of sulfuric acid solution at room temperature, which was around 25 degrees Celsius.

2.6 Planting Method

2.6.1 First generation (M1)

Seeds of each treatment were planted in separate experimental plots measuring (2 x 2 meters), divided into 5 rows. The spacing between rows was 40 cm, and the spacing between plants within each row was 20 cm. Service alleys were left between adjacent experimental plots at a distance of 50 cm. At the end of the season, the best plants showing positive changes in phenological, morphological, or productive traits compared to the control were selected and their seeds were individually preserved for cultivation in the following year to obtain M2 plants. (It is worth noting that seeds of all M1 plants were preserved when the remaining number of plants was very low). Additionally, a sample of seeds was planted without mutagen treatment as a control (C0) following the method outlined by Biswas et al., [18].

2.6.2 Second generation (M2)

In the second generation, seeds of the selected plants from the first generation were planted in three replicates in a line planting method for each treatment independently, distinguishing them from the rest of the treatments (where each line represents an independent lineage). Each line was 1 meter long with a spacing of 50 cm between each pair of lines, and an average of 20 seeds were planted per line. Additionally, three rows of the control were planted for each treatment for comparison. Selection for mutant plants, showing strong genetic isolations, was applied at the end of the year, with only 5-10% of M2 plants selected (as most mutations were not beneficial) based on the best morphological and productive traits for cultivation in the third season to obtain M3 plants.

2.6.3 Post-planting operations

Two harrowing operations were conducted in the early stages of plant growth, and weeding was carried out as needed. In addition, pest

prevention and control measures were implemented. Seeds were irrigated at planting and three days after planting, then lightly irrigated after a week. The remaining irrigations were divided with an irrigation frequency of every 7-10 days depending on the need and climatic conditions, aiming to achieve plant maturity.

2.6.4 Environmental conditions

The soil at the research site (Table 2) is characterized by its silty clay texture, with a slightly alkaline pH. It is rich in total carbonates, low in salinity and rich in absorbable phosphorus. It has a moderate potassium content and is suitable for the growth and development of soybean crops.

Weather conditions were monitored and recorded during the research period. Temperature extremes, both low and high, did not reach levels that would inhibit growth, making them suitable for the cultivation and growth of soybeans in their various varieties. However, rainfall amounts were insufficient, necessitating multiple irrigations throughout the research period, approximately one irrigation every ten days, based on the temperature.

2.7 The Studied Measurements and Readings Include

-Plant height (cm) and number of main branches.

Leaf area per cm2 by determining the area of a single leaf using the formula: leaf length × leaf width × correction factor for soybeans (0.583). Leaf area = area of a single leaf × number of leaflets on the leaf [19].

Plant leaf area = area of a single leaf × number of leaves on the plant [19].

-Weight of 100 seeds (g), number of pods per plant, seed weight per plant (g), and protein percentage (%) using the Kjeldahl method (AOAC, 1990). Protein percentage = volume (in milliliters) of consumed acid for titration × 6.25 × 100 / sample weight.

2.8 Statistical Analysis

Statistical analysis was conducted using appropriate statistical software programs (Excel and SPSS20) to perform analysis of variance (ANOVA) and determine the least significant difference for the studied traits in the selected plants from each treatment.

3. RESULTS AND DISCUSSION

-The effect of different concentrations of the mutagen NaN3 and soaking durations on some genetic varieties of soybeans in terms of plant height (cm): Table (4) indicates significant differences among soybean varieties treated with different concentrations of the mutagen NaN3 and soaking durations, and their interactions, in terms of plant height (cm).

-Genotypic Effect: was evident with the Sb337 variety surpassing in plant height (55.09 cm) compared to varieties Sb44 and Sb239 (55.01–51.29 cm).

- Soaking Duration Effect: Table 4 shows a decrease in plant height (cm) with an increase in soaking duration of seeds from (4 - 8 - 12 hours), ranging from (61.31 - 53.35 - 46.73 cm) respectively. This is consistent with the

Findings of Hajduch et al., [16] who also observed a decrease in plant height with an increase in soaking duration of seeds treated with the mutagen.

Mutagen Concentration Effect: Additionally Table (4) indicates a decrease in plant height (cm) with an increase in mutagen concentration from (1 – 2-3 – 4 mM), with percentage reductions of (2.43 – 20.24 – 39.02 – 64.63%) respectively, compared to the control (77.26 cm). Our findings align with the research of Ahire et al., [20] demonstrating statistically significant differences among all the genetic varieties treated with sodium azide in plant height. NARC-2 and AJMERI-2 exhibited increases of 26.99 cm and 26.933 cm, respectively. This leads to the conclusion that plant height decreases with higher concentrations of sodium azide.

Genotypic x Mutagen Concentration Interaction: The same table also indicates significant differences between the pairwise interactions in their impact on the studied trait. The highest values were observed in interactions between the Sb337 variety and mutagen concentration (1 mM), where the plant height reached 87.61 cm.

Genotypic x Soaking Duration Interaction: Table (4) indicates significant differences in the pairwise interactions concerning the studied trait, where the highest values were observed for the interaction between the variety Sb 44 and the soaking duration of seeds (4 hours), reaching a plant height of (62.49 cm).

Table 1. illustrates some phenological, morphological, and productivity specifications of three genotypes of soybeans

Genotypes	Germination rate %	Number of days to flowering	Number of days to maturity	Average plant height (cm)	Weight of 100 seeds (g)	Seed productivity Kg/h
Sb44	83	55	120	69	15.6	3982
Sb337	77	59	126	73	19.1	3682
Sb239	90	49	118	58	17.3	2564

Table 2. Results of soil analysis at the experiment site

Depth/cm	Physical analysis			The absorbable elements			Chemical analysis			
	%sand	%silt	%clay	N PPM Metallic	P PPM	K PPM	N%	CaCO3%	EC 5:1 milimoles/ cm	pH 5:1
30-0	179	39	44	5.7	21.4	184	0.15	31.3	0.29	7.46
Description	-	-	Silty clay	Poor	Rich	medium	medium	high	low	Light basal

Table 3. Prevailing climatic conditions at the study site during the season

Month	Rainfall/mm	Temperature/Celsius		
		Max	Min	Average
April	25	26.8	18.5	22.65
May	28	26.3	19.2	22.75
June	20	29.8	20.3	25.05
July	0	30.2	25.8	28
August	0	34.8	26.9	30.85

Latakia Meteorological Station (Bouake).

Table 4. Effect of Sodium Azide Concentrations and Seed Soaking Duration on Some Genotypes of Soybean in Plant Height (cm)

Genotype	Mutagen Concentration (NaN3)	Soaking Duration / h			Genotype X Mutagen Concentration
		4	8	12	
Sb44	0	73.70	-	=	73.70
	1	80.40	75.90	72.90	76.4
	2	64.37	61.78	59.19	61.78
	3	62.34	52.78	37.50	50.87
	4	42.85	26.56	23.50	30.97
Sb239	0	75.31	-	-	75.31
	1	79.07	74.17	67.01	73.42
	2	62.66	60.64	59.51	60.94
	3	57.82	46.92	29.45	44.73
	4	36.63	23.1	18.6	26.11
Sb337	0	82.77	-	-	82.77
	1	92.01	87.76	83.07	87.61
	2	64.46	63.38	58.6	62.15
	3	55.61	46.44	35.17	45.74
	4	37.55	20.82	16.3	24.89
$LSD_{(0.05)}$	7.34				5.76
Soaking Duration Rate /h		61.31	53.35	46.73	Genotypic Effect Rate
$LSD_{(0.05)}$	4.82				
Genotype	Sb44	62.49	54.26	48.27	55.01
X Soaking	Sb239	59.05	51.21	43.64	51.29
Duration/ h	Sb337	62.41	54.60	48.29	55.09
$LSD_{(0.05)}$	4.1				3.2
					Mutagen Concentration Rate
Mutagen	0	77.26	-	-	77.26
Concentrati	1	83.83	79.27	74.33	79.143
on X	2	63.83	61.93	59.10	61.62
Soaking	3	58.59	48.71	34.04	47.11
Duration	4	39.01	23.49	19.47	27.32
Rate/ h					
$LSD_{(0.05)}$	8.50				7.30

Mutagen Concentration x Soaking Duration Interaction: Table (4) indicates significant differences in the pairwise interactions concerning the studied trait, where the highest values were observed for the interaction between the mutagen concentration (1 mM) and the soaking duration of seeds (4 hours), reaching a plant height of (83.83 cm).

Genotypic x Mutagen Concentration x Soaking Duration Interaction: Significant differences in the triple interactions concerning the studied trait are observed in Table (4), where the highest values were recorded for the interaction between the strain 337Sb, mutagen concentration (1 Mm), and soaking duration of seeds (4 hours), resulting in a plant height of

(92.01 cm), surpassing all the studied treatments. This is consistent with the findings of Romero et al., [21] who confirmed that increasing radiation doses led to a decrease in plant height in most experiments. Additionally, Cheng et al*.,* [22] attributed the decrease in the height of mutated plants to divisions resulting from mutagenesis and the expression of genes involved in plant hormone synthesis.

The effect of sodium azide concentrations and soaking duration of seeds on some genotypes of soybeans in the trait of branching number: Although the trait of branching number in plants is a hereditary trait for the variety, it is significantly influenced by environmental factors such as photoperiod and temperature [23] hence, it was studied.

Table (5) indicates significant differences in soybean varieties treated with different concentrations of the mutagen sodium azide and soaking duration of seeds, and their interactions, in the trait of branching number in plants.

Genotypic Effect: Wherein, regarding the branching number trait, the Sb 44 variety (3.52) outperforms the 239 Sb and 337 Sb varieties (3.04–3.05), respectively, in ascending order.

Soaking Duration Effect: Table (5) demonstrates a decrease in plant branching number with an increase in seed soaking duration from (4 - 8 - 12 hours), ranging from (3.56 - 3.39 - 2.66) respectively. This aligns with findings by Horn et al*.,* [24] regarding a decrease in branching number with increased seed soaking duration for mutant seeds.

Mutagen Concentration Effect: Additionally, Table (5) illustrates a decrease in plant branching number with an increase in mutagen concentration from (1 - 2 - 3 - 4 mM), with percentage reductions of (9.06 - 5.76 - 18.13 - 33.24%) respectively, compared to the control (3.64). This aligns with the findings of Karthika and Lakshmi, [25] in their study on the effect of gamma rays on the branching number trait in soybean, highlighting its positive role in increasing plant branching number at low concentrations.

Genotypic x Mutagen Concentration Interaction: The same table also indicates significant differences between the binary interactions in their effect on the studied trait, with the highest values observed for interactions between the Sb 337 variety and mutagen concentration (1 mM), resulting in a stability branching number of (4.21).

Genotypic x Soaking Duration Interaction: Table (5) indicates significant differences between the binary interactions in their impact on the studied trait, with the highest values observed for interactions between the Sb 44 variety and soaking duration (4 hours), resulting in a plant's branching number of (3.86).

Mutagen Concentration x Soaking Duration Interaction: Table (5) indicates significant differences between the binary interactions in their impact on the studied trait, with the highest values observed for interactions between the mutagen concentration (1 mM) and soaking duration (8 hours), resulting in a plant's branching number of (4.28).

Genotypic x Mutagen Concentration x Soaking Duration Interaction: Table (5) reveals significant differences among the triple interactions in their impact on the studied trait, with the highest values observed for interactions between the genotype 337 Sb, mutagen concentration (1 mM), and soaking duration (4 hours), resulting in a plant's branching number of (4.4), surpassing all other treatments. This increase in branching in mutagen-treated plants was attributed by Hanan et al*.,* [26] to rapid cell division, elongation, and synthesis of plant proteins or nucleic acids.

Effect of Sodium Azide Concentrations and Seed Soaking Duration on Some Genotypes of Soybean in Leaf Area per Plant (cm^2): Table 6 indicates significant differences in soybean varieties treated with different concentrations of the sodium azide mutagen and seed soaking durations and their interactions in the trait of leaf area per plant.

Genotypic Effect: The genotype Sb239 (1329.11 cm2) excelled in leaf area per plant compared to the varieties 44 Sb and 337 Sb (981.84– 1086.92 cm2) in sequence.

Soaking Duration Effect: Table 6 shows a decrease in leaf area per plant with an increase in seed soaking duration from (4 – 8-12 hours), ranging from (1797.96 – 953.49– 646.42 cm2) in sequence.

Mutagen Concentration Effect: Similarly, Table 6 shows a decrease in leaf area per plant with an increase in mutagen concentration from (1 – 2-3 – 4 mM), with percentages of (30.37– 49.43– 67.02– 76.08 %) respectively, compared to the control (2558.28 cm2).

Genotypic x Mutagen Concentration Interaction: The table indicates significant differences between the pairwise interactions in their effect on the studied trait, where the highest values came from interactions between the Sb 239 genotype and mutagen concentration (mM1), reaching a leaf area per plant of (1975) cm2.

Genotypic x Soaking Duration Interaction: Table 6 indicates significant differences between the pairwise interactions in their effect on the studied trait, where the highest values came from interactions between the Sb 239 genotype and soaking duration (4 hours), reaching a leaf area per plant of (2005.92) cm2.

Mutagen Concentration x Soaking Duration Interaction: Table 6 indicates significant differences between the pairwise interactions in their effect on the studied trait, where the highest values came from interactions between mutagen concentration (1 mM) and soaking duration (4 hours), reaching a leaf area per plant of (2826.71) cm2.

Table 5. Effect of sodium azide concentrations and seed soaking duration on some soybean genotypes in branching trait

Genotype	Mutagen Concentration (NaN3)	Soaking Duration/h			Genotype x Mutagen Concentration
		4	8	12	
Sb44	0	3.73	-	-	3.73
	1	4.15	5.00	3.00	4.05
	2	4.33	4.00	3.50	3.94
	3	3.44	3.33	3.00	3.26
	4	3.50	3.00	2.00	2.83
Sb239	0	3.54	-	-	3.54
	1	4.09	3.6	3.28	3.66
	2	3.37	3.2	3.16	3.24
	3	3.14	3	3	3.05
	4	3	2.66	1	2.22
Sb337	0	3.66	-	-	3.66
	1	4.4	4.25	3.97	4.21
	2	3.2	3.07	3	3.09
	3	3.14	2.78	2	2.64
	4	3	2.75	1	2.25
$LSD_{(0.05)}$	0.98				0.86
Soaking Duration Rate / h		3.56	3.39	2.66	Genotypic Effect Rate
$LSD_{(0.05)}$	0.74				
Genotype x Soaking Duration / h	Sb44	3.86	3.83	2.88	3.52
	Sb239	3.40	3.11	2.61	3.04
	Sb337	3.44	3.21	2.49	3.05
$LSD_{(0.05)}$	0.91				0.63
					Mutagen Concentration Rate
Mutagen Concentration x Soaking Duration / h	0	3.64	-	-	3.64
	1	4.21	4.28	3.42	3.97
	2	3.63	3.42	3.22	3.43
	3	3.24	3.04	2.67	2.98
	4	3.17	2.80	1.33	2.43
$LSD_{(0.05)}$	0.68				0.54

Table 6. Effect of Sodium Azide Concentrations and Seed Soaking Duration on Some Soybean Genotypes in Leaf Area per Plant Trait (cm2)

Genotype	Mutagen Concentration (NaN3)	Soaking Duration/h			Genotype x Mutagen Concentration
		4	8	12	
Sb44	0	1950.03	-	-	1950.03
	1	2211.20	1264.81	1108.76	1528.25
	2	1422.81	1114.41	841.51	1126.24
	3	1514.44	684.15	243.98	814.19
	4	713.59	449.49	213.02	458.70
Sb239	0	2852.25	-	-	2852.25
	1	3179.50	1577.59	1170.20	1975.77
	2	2309.33	1006.97	937.46	1417.92
	3	1427.43	1199.81	564.48	1063.91
	4	1107.40	881.49	587.66	858.85
Sb337	0	2872.55	-	-	2872.58
	1	3089.43	1452.11	978.01	1839.85
	2	2334.91	1078.29	597.83	1337.01
	3	1083.13	486.22	388.27	652.54
	4	1182.32	246.61	125.92	518.87
$LSD_{(0.05)}$	301.9				310.3
Soaking Duration Rate / h		1797.96	953.49	646.42	Genotypic Effect Rate
$LSD_{(0.05)}$	258.5				
Genotype x Soaking Duration / h	Sb44	1465.51	878.21	601.81	981.84
	Sb239	2005.92	1166.47	814.95	1329.11
	Sb337	1922.51	815.81	522.51	1086.92
$LSD_{(0.05)}$	284.1				195.3
					Mutagen Concentration Rate
Mutagen Concentration x Soaking Duration / h	0	2558.28	-	-	2558.28
	1	2826.71	1431.51	1085.66	1781.29
	2	2022.35	1066.56	792.27	1293.72
	3	1341.66	790.06	398.91	843.54
	4	1001.11	525.86	308.87	611.94
$LSD_{(0.05)}$	401.02				319.4

Genotypic x Mutagen Concentration x Soaking Duration Interaction: The table shows significant differences between the triple interactions in their effect on the studied trait, where the highest values came from interactions between the genotype 239 Sb, mutagen concentration (1 mM), and soaking duration (4 hours), reaching a leaf area per plant of (3179.50) cm2, surpassing all the studied treatments.

Effect of Sodium Azide Concentrations and Seed Soaking Duration on Some Genotypes of Soybean in the Trait of Pod Number: Table 7 indicates significant differences in soybean varieties treated with different concentrations of the sodium azide mutagen and seed soaking durations and their interactions in the trait of pod number per plant.

Genotypic Effect: The genotype Sb239 (124.71 pods) excelled in pod number per plant compared to the varieties Sb44 and 337 Sb (110.15– 73.87 pods) in sequence.

Soaking Duration Effect: Table 7 shows a decrease in pod number per plant with an increase in seed soaking duration from (4 – 8-12 hours), ranging from (120.37 – 100.24– 88.12 pods) in sequence. This is consistent with Horn et al. [24] who reported a decrease in pod number with increased seed soaking duration in seeds treated with the mutagen.

Mutagen Concentration Effect: Similarly, Table 7 shows a decrease in pod number per plant with an increase in mutagen concentration from (1 – 2-3 – 4 mM), with percentages of (12.02– 3.83 – 26.25– 55.92%) respectively, compared to the control (123.36 pods). These results align with

Karthika and Lakshmi, [25] who emphasized the positive role of gamma rays in increasing pod number per plant.

Genotypic x Mutagen Concentration Interaction: Table 7 indicates significant differences between the pairwise interactions in their effect on the studied trait, where the highest values came from interactions between the genotype Sb44 and mutagen concentration (mM1), reaching a stable pod number of (150.31).

Our results are consistent with Essel, [27] who found significant high results due to the interaction of genotypes and treatment with different concentrations of sodium azide in the trait of pod number per plant, where the pod number increased for each plant at a dose of 0.04% over 0.06% SA, indicating an increase in pod number in genotypes treated with the sodium azide mutagen compared to the control and increasing with increasing mutagen concentration.

Genotypic x Soaking Duration Interaction: Table 7 indicates significant differences between the pairwise interactions in their effect on the studied trait, where the highest values came from interactions between the genotype Sb239 and soaking duration (4 hours), reaching a pod number per plant of (142.84).

Table 7. Effect of Sodium Azide Concentrations and Seed Soaking Duration on Some Soybean Genotypes in Number of Pods Trait

Genotype	Mutagen Concentration (NaN3)	Soaking Duration/h			Genotype x Mutagen Concentration
		4	8	12	
Sb44	0	107.60	-	-	107.60
	1	181.92	135.00	134.00	150.31
	2	150.50	140.22	127.33	139.35
	3	135.40	118.30	89.50	114.40
	4	56.00	35.66	18.00	36.55
Sb239	0	142.08	-	-	142.08
	1	160.60	144.00	137.50	147.37
	2	156.75	135.40	134.50	142.22
	3	147.00	121.25	79.50	115.92
	4	107.00	96.00	77.00	93.33
Sb337	0	120.41	-	-	120.41
	1	126.70	114.06	110.06	116.94
	2	122.09	98.04	88.04	102.72
	3	53.00	38.90	35.90	42.60
	4	47.50	26.08	26.08	33.22
$LSD_{(0.05)}$	9.1				8.5
Soaking Duration Rate / h		120.37	100.24	88.12	Genotypic Effect Rate
$LSD_{(0.05)}$	8.70				
Genotype x	Sb44	130.96	107.29	92.21	110.15
Soaking Duration /	Sb239	142.84	124.16	107.13	124.71
h	Sb337	87.32	69.27	65.02	73.87
$LSD_{(0.05)}$	7.3				6.50
					Mutagen Concentration Rate
Mutagen	0	123.36	-	-	123.36
Concentration x	1	156.41	131.02	127.17	138.20
Soaking Duration	2	143.11	124.55	116.62	128.09
/ h	3	111.80	92.82	68.30	90.97
	4	70.17	52.58	40.36	54.37
$LSD_{(0.05)}$	6.70				8.10

Mutagen Concentration x Soaking Duration Interaction: Table 7 indicates significant differences between the pairwise interactions in their effect on the studied trait, where the highest values came from interactions between mutagen concentration (1 mM) and soaking duration (4 hours), reaching a pod number per plant of (156.41).

Genotypic x Mutagen Concentration x Soaking Duration Interaction: The table shows significant differences between the triple interactions in their effect on the studied trait, where the highest values came from interactions between the genotype Sb44, mutagen concentration (1 mM), and soaking duration (4 hours), reaching a pod number per plant of (181.92), surpassing all the studied treatments.

Our results are in line with Kumar et al., [28] in their study on gamma irradiation of beans, where they observed that low and medium doses led to an increase in the average pod number per plant, an important trait for plant breeders, and suggested that this could be attributed to the physiological effects of low and medium mutagen doses and their hydrolysis products on the increasing number of pods.

Effect of Sodium Azide Concentrations and Seed Soaking Duration on Some Genotypes of Soybean in the Trait of 100-Seed Weight (g): Table 8 indicates significant differences in soybean varieties treated with different concentrations of the sodium azide mutagen and seed soaking durations and their interactions in the trait of 100-seed weight.

Genotypic Effect: The genotype Sb239 (14.15 g) excelled in 100-seed weight compared to the varieties Sb44 and 337 Sb (12.51– 12.85 g) in sequence.

Soaking Duration Effect: Table 8 shows a decrease in 100-seed weight with an increase in seed soaking duration from (4 – 8-12 hours), ranging from (14.90– 12.98– 11.64 g) in sequence.

Mutagen Concentration Effect: Similarly, Table 8 shows a decrease in 100-seed weight with an increase in mutagen concentration from (1 – 2-3 – 4 mM), with percentages of (1.93– 19.14– 29.80–52.76%) respectively, compared to the control (17.55 g). Our results fully agree with Oderigah et al*.,* [17] and Kumar et al., [28] regarding the increase in 100-seed weight in plants treated with low and medium doses.

Genotypic x Mutagen Concentration Interaction: The table indicates significant differences between the pairwise interactions in their effect on the studied trait, where the highest values came from interactions between the genotype Sb44 and mutagen concentration (mM1), reaching a 100-seed weight of (19.04 g). For a 24-hour duration, it reached (20.1 g). This may be attributed to the mutagen's effect on plant hormones [18] leading to an increase in 100-seed weight. This aligns with Ibrahim et al., [29].

Genotypic x Soaking Duration Interaction: Table 8 indicates significant differences between the pairwise interactions in their effect on the studied trait, where the highest values came from interactions between the genotype Sb239 and soaking duration (4 hours), reaching a 100-seed weight of (15.26 g).

Mutagen Concentration x Soaking Duration Interaction: Table 8 indicates significant differences between the pairwise interactions in their effect on the studied trait, where the highest values came from interactions between mutagen concentration (1 mM) and soaking duration (4 hours), reaching a 100-seed weight of (18.76 g).

Genotypic x Mutagen Concentration x Soaking Duration Interaction: The table shows significant differences between the triple interactions in their effect on the studied trait, where the highest values came from interactions between the genotype Sb44, mutagen concentration (1 mM), and soaking duration (4 hours), reaching a 100-seed weight of (19.74 g), surpassing all the studied treatments. This aligns with similar studies on the effect of radiation on soybean plants, confirming the mutagens' effect in increasing 100-seed weight and inducing genetic variation [30].

Effect of Sodium Azide Concentrations and Seed Soaking Duration on Some Genotypes of Soybean in the Trait of Seed Weight per Plant (g): Table 9 shows significant differences in soybean varieties treated with different concentrations of sodium azide mutagen, seed soaking durations, and their interactions in the trait of seed weight per plant [31].

Genotypic Effect: The genotype Sb44 (23.96 g) excelled in seed weight per plant compared to the varieties Sb239 and 337 Sb (23.24– 15.13 g) in sequence.

Soaking Duration Effect: Table 9 indicates a decrease in seed weight per plant with an

increase in seed soaking duration from (4 – 8-12 hours), ranging from (26.59– 19.77– 15.97 g) in sequence.

Mutagen Concentration Effect: Similarly, Table 9 shows a decrease in seed weight per plant with an increase in mutagen concentration from (1 – 2-3 – 4 mM), with percentages of (17.04– 22.64– 53.66– 77.83%) respectively, compared to the control (31.62 g).

Genotypic x Mutagen Concentration Interaction: Significant differences exist between the pairwise interactions, with the highest values coming from interactions between the genotype Sb44 and mutagen concentration (mM1), reaching a seed weight per plant of (43.86 g).

Genotypic x Soaking Duration Interaction: Table 9 indicates significant differences between the pairwise interactions, with the highest values coming from interactions between the genotype Sb44 and soaking duration (4 hours), reaching a seed weight per plant of (31.02 g).

Mutagen Concentration x Soaking Duration Interaction: Significant differences exist between the pairwise interactions, with the highest values coming from interactions between mutagen concentration (1 mM) and soaking duration (4 hours), reaching a seed weight per plant of (44.08 g).

Table 8. Effect of Sodium Azide Concentrations and Seed Soaking Duration on Some Soybean Genotypes in 100g-Seed Weight Trait

Genotype	Mutagen Concentration (NaN3)	Soaking Duration/h			Genotype x Mutagen Concentration
		4	8	12	
Sb44	0	18.50	-	-	18.50
	1	19.74	19.07	18.33	19.04
	2	15.92	10.55	8.88	11.78
	3	11.85	9.44	9.16	10.15
	4	10.00	8.88	8.33	9.07
Sb239	0	17.19	-	-	17.19
	1	18.01	17.79	16.03	17.27
	2	17.42	16.56	15.10	16.36
	3	16.96	16.27	14.77	16.00
	4	8.65	6.60	5.74	6.99
Sb337	0	16.95	-	-	16.95
	1	18.55	17.74	15.80	17.36
	2	18.30	13.41	11.61	14.44
	3	12.12	10.66	9.66	10.81
	4	11.33	8.80	6.33	8.82
$LSD_{(0.05)}$	1.23				1.13
Soaking Duration Rate / h		14.90	12.98	11.64	Genotypic Effect Rate
$LSD_{(0.05)}$	1.19				
Genotype x Soaking Duration / h	Sb44	14.37	11.98	11.17	12.51
	Sb239	15.26	14.305	12.91	14.15
	Sb337	15.07	12.65	10.85	12.85
$LSD_{(0.05)}$	2.10				1.70
					Mutagen Concentration Rate
Mutagen Concentration x Soaking Duration / h	0	17.55	-	-	17.55
	1	18.76	18.20	16.72	17.89
	2	17.21	13.51	11.86	14.19
	3	13.64	12.12	11.19	12.32
	4	9.99	8.09	6.80	8.29
$LSD_{(0.05)}$	1.92				1.75

Genotypic x Mutagen Concentration x Soaking Duration Interaction: The table shows significant differences between the triple interactions, with the highest values coming from interactions between the genotype Sb44, mutagen concentration (1 mM), and soaking duration (4 hours), reaching a seed weight per plant of (54.88 g), surpassing all the studied treatments. The increase in weight at concentration C1 (1 mM) may be attributed to the mutagen's stimulation of plant metabolism [18]. The decrease in seed weight may be due to sodium azide inhibiting a hormone, leading to decreased productivity components such as seed weight per plant. Additionally, an increase in hydrolysis of complex compounds like starch may occur, along with increased degradation of RNA and nucleotides in nucleic acids [29] ultimately affecting productivity traits. These results align with findings by Oderigah et al., [14].

Effect of Sodium Azide Concentrations and Seed Soaking Duration on Some Genotypes of Soybean in the Trait of Protein Percentage (%): Table 10 indicates significant differences in soybean varieties treated with different concentrations of sodium azide mutagen, seed soaking durations, and their interactions in the trait of protein percentage per plant.

Genotypic Effect: The genotype Sb44 (28.83%) excelled in protein percentage per plant compared to the varieties Sb239 and 337 Sb (28.26– 28.49%) in sequence.

Table 9. Effect of Sodium Azide Concentrations and Seed Soaking Duration on Some Soybean Genotypes in Seed Weight per Plant Trait

Genotype	Mutagen Concentration (NaN3)	Soaking Duration/h			Genotype x Mutagen Concentration
		4	8	12	
Sb44	0	32.46	-	-	32.46
	1	54.88	39.51	37.20	43.86
	2	34.45	23.40	18.20	25.35
	3	24.35	17.95	14.35	18.88
	4	10.40	7.34	5.60	7.78
Sb239	0	32.31	-	-	32.31
	1	40.64	35.14	28.47	34.75
	2	34.75	26.38	22.81	27.98
	3	29.43	19.74	10.07	19.74
	4	12.02	11.8	7.7	10.51
Sb337	0	30.10	-	-	30.10
	1	36.74	31.93	28.61	32.43
	2	29.30	17.64	13.21	20.05
	3	7.42	4.66	3.94	5.34
	4	4.75	1.82	1.56	2.71
$LSD_{(0.05)}$	3.90				3.62
Soaking Duration Rate / h		26.59	19.77	15.97	Genotypic Effect Rate
$LSD_{(0.05)}$	2.76				
Genotype x Soaking Duration / h	Sb44	31.02	22.05	18.8375	23.96
	Sb239	29.21	23.26	17.2625	23.24
	Sb337	19.55	14.01	11.83385	15.13
$LSD_{(0.05)}$	3,10				4.20
					Mutagen Concentration Rate
Mutagen Concentration x Soaking Duration / h	0	31.62	-	-	31.62
	1	44.08	35.52	31.42	37.01
	2	32.83	22.47	18.07	24.46
	3	20.40	14.11	9.45	14.65
	4	9.05	6.98	4.95	7.01
$LSD_{(0.05)}$	2.98				3.30

Table 10. Effect of Sodium Azide Concentrations and Seed Soaking Duration on Some Soybean Genotypes in Protein Percentage Trait

Genotype	Mutagen Concentration (NaN3)	Soaking Duration/h			Genotype x Mutagen Concentration
		4	8	12	
Sb44	0	28.53	-	-	28.53
	1	32.76	29.84	28.61	30.40333
	2	31.38	28.81	27.23	29.14
	3	30.84	27.84	26.80	28.49333
	4	29.23	26.53	25.92	27.22667
Sb239	0	29.61	-	-	29.61
	1	33.30	32.15	32.23	32.56
	2	30.76	28.69	26.15	28.53333
	3	29.69	26.76	23.92	26.79
	4	27.84	24.69	23	25.17667
Sb337	0	29.30	-	-	29.30
	1	31.61	30.38	29.38	30.45667
	2	30.84	28.46	26.92	28.74
	3	30.07	27.00	25.46	27.51
	4	28.46	25.53	23.92	25.97
$LSD_{(0.05)}$	3.2				2.8
Soaking Duration Rate / h		30.56	28.05	26.62	Genotypic Effect Rate
$LSD_{(0.05)}$	2.3				
Genotype x Soaking Duration / h	Sb44	31.27	28.22	27.02	28.83
	Sb239	30.45	28.12	26.22	28.26
	Sb337	30.80	28.12	26.57	28.49
$LSD_{(0.05)}$	2.9				1.3
					Mutagen Concentration Rate
Mutagen Concentration x Soaking Duration / h	0	29.23	-	-	29.23
	1	32.92	30.90	30.10	31.31
	2	31.15	28.72	26.76	28.87
	3	30.32	27.42	25.40	27.71
	4	28.97	25.58	24.16	26.24
$LSD_{(0.05)}$	3.3				2.4

Soaking Duration Effect: Table 10 shows a decrease in protein percentage per plant with an increase in seed soaking duration from (4 – 8-12 hours), ranging from (30.56– 28.05– 26.62%) in sequence.

Mutagen Concentration Effect: Similarly, Table 10 shows a decrease in protein percentage per plant with an increase in mutagen concentration from (1 – 2-3 – 4 mM), with percentages of (7.11– 1.23– 5.20–10.22%) respectively, compared to the control (29.23%).

Genotypic x Mutagen Concentration Interaction: Significant differences exist between the pairwise interactions, with the highest values coming from interactions between the genotype Sb337 and mutagen concentration (mM1), reaching a protein percentage per plant of (30.45%).

Genotypic x Soaking Duration Interaction: Table 10 indicates significant differences between the pairwise interactions, with the highest values coming from interactions between the genotype Sb44 and soaking duration (4 hours), reaching a protein percentage per plant of (31.27%).

Mutagen Concentration x Soaking Duration Interaction: Significant differences exist between the pairwise interactions, with the highest values coming from interactions between mutagen concentration (1 mM) and soaking duration (4 hours), reaching a protein percentage per plant of (32.92%)

Genotypic x Mutagen Concentration x Soaking Duration Interaction: The table shows significant differences between the triple interactions, with the highest values coming from interactions between the genotype Sb239, mutagen concentration (1 mM), and soaking duration (4 hours), reaching a protein percentage per plant of (33.30%), surpassing all the studied treatments.

4. CONCLUSIONS AND RECOMMENDA-TIONS

4.1 Conclusions

- The experiment concludes that sodium azide, as a chemical mutagen with high mutagenic potential, induces variations, possibly hereditary, when treating soybean seeds. This provides plant breeders with a wider range of options for selecting favorable traits from early stages of growth.
- Different concentrations of sodium azide, along with varying soaking durations, resulted in significant changes in numerous morphological, physiological, productive, and qualitative traits among the studied genotypes.
- Concentration C1 (1mM) of sodium azide exhibited the greatest positive impact and stimulation on most of the studied traits, leading to the selection of several plants.
- A significant decrease was observed in most studied traits with increasing soaking durations from T1 to T3 hours.
- A gradual decrease in most studied traits among the genotypes was observed with increasing mutagen concentrations and soaking durations within each concentration. The best interactions were found at concentration C1 (1mM) and time T1 (4 hours of soaking).

4.2 Recommendations

1- It is recommended to use the chemical mutagen sodium azide for mutagenizing various crop plants, employing low concentrations and short soaking durations for the seeds.

2- Continuation of work on selected plants for several mutagenic generations is advised to achieve superior hereditary genotypes in various traits, especially production and productivity components.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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