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USE OF HYDROXYLAMINE(NH2OH) TO INDUCE GENETIC CHANGES IN SOME LABORATORY AND FIELD TRAITS IN BLACK BEAN PLANT PHASEOLUS VULGARIS

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Abstract:

The study included the creation of variations using the chemical mutagenic hydroxylamine(NH2OH)in four concentrations 0, 0.5 ,1, 1.5 M of black bean plant Phaseolus vulgaris . If the seeds were soaked in the above concentrations for a different period of time15, 24, 32H. The seeds treated with the mutagen were divided into two parts: the first part was sown in Petri dishes, while the second part was planted in the field according to the randomized complete block design. Germination percentage, germination speed/day, and root length were calculated in the laboratory. When the plants reached the stage of maturity, random samples were taken from the plants to study the effect of hydroxylamine NH2OH on the yield components, namely the number of pods, the length of the pod, and the number of seeds / pod. The results showed that hydroxylamineNH2OH had a significant effect in reducing the percentage of germination, germination speed / day, average root length, average pod length, number of pods / plant and number of seeds / pod at high concentrations. It turns out that the best concentration of the mutagen(1M) to cause beneficial changes can be selected to increase the yield of the plant.

Keywords: legumes, Phaseolus vulgaris , seeds, chemical mutagen, mutation, hydroxyl amine

INTRODUCTION

Phaseolus vulgaris black bean belongs to the legume family. It is characterized by its high content of vegetable protein and fiber that helps digestion and fights constipation (1). In a new study, it was confirmed that black beans contain the highest percentage of antioxidants that protect against heart disease and cancer (2).

Black beans help lower blood cholesterol and high blood pressure. The saponins present in black beans act as antioxidants that have the ability to lower cholesterol. Bean fibers are important for reducing total cholesterol and triglyceride levels (3). In addition, it has the ability to lower blood sugar (4).

Therefore, it is necessary to search for advanced scientific methods for breeding and improving black beans in order to devise new varieties with good agricultural specifications by following modern methods of creating mutations using physical or chemical mutagens (5).And since the use of mutagens increases the frequency of mutations by hundreds of times over the normal situation in all neighborhoods. Therefore, mutagens have been used in many scientific researches in the field of raising agricultural crops (6).

One of the most used chemicals to create mutations is hydroxyl amine (NH2OH), which is considered an important mutagenic because it disrupts DNA replication, causing nucleotides to transfer from one purine to another or pyrimidine to another.(7) And its effect starts from the stage of germination, It was used to improve the characteristics of many agricultural plants and increase their yield (8).

Due to the importance of the black bean plant and the lack of studies related to improving its genetic characteristics, the hydroxyl amine was used as a chemical mutagen in order to find the best concentration through which we can obtain genetic variations that lead to improving the qualitative and quantitative characteristics.

Aim of the study :

1. Creation of genetic variations using hydroxyl amine at different time periods.
2. Determine the best concentration to generate contrast.
3 . Determine the concentrations negatively affecting the plant.

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MATERIALS AND METHODS

1. Black bean seeds obtained from the General Company for Industrial Crops / Ministry of Agriculture were used.
2. Prepare a hydroxyl amine NH2OH solution with a concentration of 1 M by dissolving 3.3 g of the mutagen powder in 50 ml of distilled water. Then came the following fears: 0.5 ,1, 1.5 M. Adjust the pH to 4 at room temperature.
3. Twenty black bean seeds were soaked in different concentrations in darkened bottles to obtain the highest frequencies of mutations (9).
4. Then the seeds were washed with distilled water and transferred to Petri dishes.
5. Petri dishes contained filter papers and were moistened with water daily. The number of seedlings was counted every day until the germination phase stopped, which lasted for four days. The percentage of germination and the speed of germination (10) were calculated. With measuring the length of roots for all seeds treated with mutagen and untreated.
6. Field experiment: It was carried out in a private nursery in Al-Mashatel Street / Al-Adhamiya / in Baghdad Governorate.
7. Plastic containers with a capacity of 6 kg were used, and the holes at the bottom of the containers were closed with bitumen and filled with mixed soil.
8. The experiment was carried out with a random block design with three replications with two factors, the first factor being the hydroxylamine(NH2OH concentration and the second factor the time period, then the L.S.D test was used at the level of probability 0.05 (11).
9. Seeds were sown in containers and agricultural operations were carried out such as irrigation and weed control when needed.
10. Add nitrogen fertilizer (urea 50%) at an amount of 4 grams / plastic container when preparing the soil (12).
11. At the end of the experiment, the length of the pod was measured using a regular ruler, the number of pods/plant, and the number of seeds per pod.
12. The experiment was carried out in a random block design with three replications:

The first: the concentration of the hydroxylamine(NH2OH with four concentrations of 0, : 0.5 ,1, 1.5 M. and its symbol T0,T1,T2,T3.

The second: the time period for soaking the seeds, which is 15, 24, 32, and its symbol Z1,Z2,Z3.

RESULTS AND DISCUSSION

Table No. (1) shows that the concentration of hydroxylamine(NH2OH had a significant effect in reducing the percentage of germination and the speed of germination, as the higher the concentration of the mutagen and the duration of soaking the seeds, the percentage of germination and the speed of germination decreased. At a concentration of 1.5 m / 32 hours, the lowest percentage of germination was recorded, reaching 30.2. A decrease was recorded in the germination speed to 1.05 compared to the control plants that gave a germination rate of 100% and a germination speed of 6.23. While the speed of germination B was recorded until it reached a concentration of 1.05 for 32 hours. Compared to control plants which gave a germination rate of 6.21. The decrease is due to the percentage and the speed of germination and the speed of germination 6.23. The reason for the decrease in the percentage of germination and the speed of germination is due to the direct effect of hydroxylamine(NH2OH on the molecular structure of DNA, as it impedes DNA replication, causing the transfer of nucleotides from one purine to another or pyrimidine to another. Which causes a disturbance in the metabolic processes within plant (13), especially with an increased seed soaking period in the dark. This is consistent with the findings of studies that the rate of germination and the speed of germination of seeds of many agricultural crops decrease when treated with chemical mutagens (14), especially when using high concentrations, which leads to the inhibition of enzymes important in the metabolism process. , such as alpha- and beta-amylase, which are involved in important processes such as respiration and starch synthesis in prodromal cells of the fetus (15).Four days after sowing the seeds in Petri dishes, the lengths of the roots of the germinated seeds treated and untreated with the chemical mutagen were measured. Table No. (2) shows the root lengths negatively affected by the mutagen and the period of soaking the seeds, until the average root length reached 45.3 at a concentration of 1.5 m / 32 hours.

The reasons for short radical lengths are due to the effect of hydroxylamine on cell division by inhibiting oxidative processes and oxidative phosphorylation that are prepared for cell division and thus the catabolism of ATP in the PROPHASE stage and thus the size of the nucleus increases and decomposes with high concentrations of mutagen (16).

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The results of Table (3)indicate that the hydroxylamine(NH2OH concentration and the time period for soaking the seeds had a significant effect on the number and length of pods/plant, as the highest rate of pod number and length reached at a concentration of 1 M/24 hours. It recorded 17 pods/plant compared to control plants. The reason is due to the effect of the mutagen on the auxin hormone, and thus on the growth rate, fertilization and pollination processes, which led to an increase in the number and length of pods at low concentrations, and this was confirmed by a group of scientists (17). As for the decrease in the rate and length of pods at a concentration of 1.5 M, the possibility of the mutagen negatively affecting the growth rate through its direct effect on DNA replication and thus a decrease in protein synthesis (18). The results also showed in Table 3 that the chemical mutagen and the soaking period of the seeds had a significant effect on the number of seeds per pod. Where the concentration was recorded as 1.5 Molar / 15 hours in reducing the number of seeds / pods (empty pods).

It is possible that a lethal mutation resulted in sterility (19). While the concentration of 1 M according to the difference of the soaking period recorded the highest mean number of seeds/pod.

The reason for the decrease in yield at a high concentration of the hydroxylamine(NH2OH mutagen is due to the inhibition of one of the hormones responsible for the rate of the number of pods in the plant and the number of seeds in the pods, or hydroxylamine in the RNA was knocked down and thus changed the composition of nucleotides in the DNA.

RECOMMENDATIONS : With this research experiment, we recommend that seeds not be soaked with chemical mutagens for more than 24 hours. Do not use high concentrations of hydroxylamine. (1 M) is preferred to produce variations useful for selection by plant breeders.

Table (1) The effect of the chemical agent and the time period of soaking the seeds on the percentage of germination and speed of germination

samples		germination %	Germination speed
T0	Z1	100	6.23
	Z2	99	6.23
	Z3	100	6.21
T1	Z1	90.8 ± 0.6 *	5.3± 0.7*
	Z2	82.4±0.6*	5.3± 0.3 *
	Z3	78.6± 1.2*	4.5± 1.2 *
T2	Z1	60.9±1.8 *	3.7±0.8 *
	Z2	51.2±1.74 *	3.2± 0.6*
	Z3	49±2.0 *	2.9± 0.6 *
T3	Z1	40.5±1.5 *	1.9± 2.6*
	Z2	35.1± 2.5 *	1.2± 1.9*
	Z3	30.2± 3.1 *	1.05±3.6 *
L.S.D		1.8	1.5

* Significant difference at the probability level of 0.05.

Table (2) The effect of the chemical agent and the time period of soaking the seeds on the average root length (mm)

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samples		Average root length (mm)
T0	Z1	94
	Z2	93
	Z3	94
T1	Z1	77.5±0.2 *
	Z2	73±0.7 *
	Z3	68.5± 0.5 *
T2	Z1	55.6± 0.5*
	Z2	54±0.8 *
	Z3	52.8±1.2 *
T3	Z1	50.3± 1.4 *
	Z2	49±2.0 *
	Z3	45.3± 2.5*
L.S.D	1.76	

* Significant difference at the probability level of 0.05.

Table (3) Effect of chemical mutagen and time period of soaking seeds on average number and length of pods / plant, Number of seeds/pod.

samples		Average number of pods/plant	Average pod length (cm).	Number of seeds/pod.
T0	Z1	12	4.5	10
	Z2	12	4.3	9
	Z3	11	4.7	10
T1	Z1	13± 0.2*	3.4±0.6 *	9 ± 0.5*
	Z2	13.7±0.2 *	3.7±0.4 *	8 ± 0.7*
	Z3	16.4± 0.9*	3.1± 1.9*	7 ± 2.3*
T2	Z1	16.8±0.8*	2.9±1.6 *	15 ±2.0 *
	Z2	17± 1.5 *	2.7±1.56 *	16 ±1.6 *
	Z3	17±2.0 *	2.3±2.1 *	16 ± 1.3*
T3	Z1	14.3± 2.6*	1.56± 1.4*	0 ±0.91 *
	Z2	13.3 ±0.9*	1.4±1.5 *	0 ± 0.8*
	Z3	13± 2.8*	1.0± 3*	0±0.5 *
L.S.D	2.2		1.5	1.57

* Significant difference at the probability level of 0.05.

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