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Article

Effect of Sodium Azide (NaN_3) on micropropagant and tolerance of Salt Stress of Strawberry cv. Albion

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ABSTRACT

Sodium azide (NaN_3) is widely used to induce mutagenesis within *in* vitro plant systems.The aim of current study was to develop mutants of Strawberry cv. albion tolerant to salinity stress. plant regeneration from runner explant cultured on multiplication Murashige and Skoog (MS) medium contain 0.5 mg l^{-1} of 6-benzyladenine (BA) and 0.1 mg l^{-1} of Naphthaleneacetic acid (NAA) and sodium azide (NaN3) as a chemical mutagens at concentrations (0.25.0.5) mM after 4 weeks , the mutagens shoots were exposed to differnet concentrations of Sodium Chloride (NaCl) (6 , 10 , 14 , 22) ds m^{-1}. The results revealed that the superiority of 6 ds m^{-1} of NaCl without mutation of sodium azide in giving the highest rate of the number of shoots reached 9.25 shoot $explant^{-1}$ and giving the highest average length of shoots,which was 3.04 cm and the highest rate of fresh weight reached 3.197 gm ,while the treatment sodium azide led to decrease in the number of shoots upon mutagenesis with NaN_3 at both concentrations and at all salt levels.A significant increasing of Sodium ion was observed in the shoots mutagenic with sodium azide at 0.5 mM and grown on a medium with 22 dS m^{-1} of NaCl, as it reached 42.90 mg g^{-1} and the highest calcium ion was 6.707 mg g^{-1} when treated with 0.25 mM of sodium azide with 6 dS m^{-1} NaCl ,It also gave the highest potassium concentration 31.50 mg g^{-1}.

Keywords: Strawberry cv. albion, microprpagation, Sodium azide, Mutagens, Salt tolerance

INTRODUCTION

Strawberry (*Fragaria* x *ananassa* Duch.) is a perennial herbaceous perennial plant belonging to the Rosaceae family. It is one of the fruits that grows in the southern hemisphere in temperate and semi-temperate regions [1] , are plants from the Rosaceae. Their ploidy types range from diploid to decaploid [2,3], while wild members of the genus distributed throughout the northern hemisphere and parts of western South America [4]. and follow the fruit with small fruits and is the fourth most consumed fruit after apples, oranges and bananas [5]. During the past few years Iraq lands had witnessed an adverse environmental stress such as saline and drought conditions which affected negatively on crop yields [6].Generally, the foremost effect of several environmental stresses, producing salt stress generates reactive oxygen/nitrogen species (ROS/RNS) and enhances oxidative stress load in plants[7].Many studies have shown the role of chemical mutagens in stimulating genetic variation in plants because of their essential characteristics for the success of the breeding program in plants that reproduce vegetatively and sexually [8]. Sodium azide NaN3 is widely used to induce mutations in plant systems in vitro , although these mutations are indirect and Sodium azide is considered relatively safe to deal with chemical mutations, is inexpensive and does not cause cancer [9]. The pH of the solution influences the mutagenic efficiency of sodium azide, and it has been shown that azide is most effective in inducing mutations at pH 3 [10]. Despite the wide range of concentrations at which sodium azide is used, the mutagen generally induces point mutation in the genome, impairing metabolic activity, growth and development, inhibiting protein synthesis and DNA replication [11]. The present study was planned to assess the genetic variations through chemical mutation using sodium azide on strawberry cv. Albion at morphological level, as well as physiological level in order to evaluate the tolerance to salt stress.

MATERIALS AND METHODS

This study was carried out during 2021 at the the Ministry of Science and Technology/ Directorate of Agricultural Research/ Genetic Engineering Department.

Plant material, in vitro methods

The uniform in vitro shoots of Strawberry cv. Albion from the multiplication stage (from the third sub culture).These shoots were treated with mutagen during the fourth subculture of the multiplication stage.

Culture medium composition

Murashige and skoog (MS) basal medium was used as a basal nutrient medium [12] The basal medium was supplemented with 0.5 mg l^{-1} 6-Amino Benzyl Purine (BA) + 1.5 mg l^{-1} Kintin (Kin) [13] as imitation medium for culturing the sterilized shoot tip runners, After 4 weeks, the shoots are cultured on the medium of the multiplication supplemented with 0.5 mg l^{-1} of BA and 0.1 mg l^{-1} of NAA. pH of the medium was adjusted to 5.7 .The cultures were incubated in a culture room under 16-h light and 8-h dark photo period at 23±1°C.

Mutagen application

In the fourth subculture of mutant Sodium Azide (NaN3) were added after they were Cold sterilization of two mutagenic concentrations. Uniform multiplied shoot explants were longitudinally dissected and dipped into mutagenic solution at (0.25, 0.5) mM for 30 min in addition to the control treatment which was left without dipping in any mutagens. Then the explants were washed with sterile distilled water for three times.Then the explants were cultured in the nutrient medium for multiplication.Individual shoots were separated and transferred into the multipling MS basal medium supplemented with 0.5 mg l^{-1} of BA and 0.1 mg l^{-1} of NAA

Salinity treatment: The mutagenic and non-mutagenic shoots were grown on MS medium containing 0.5 mg. l^{-1} of BA and 0.1 mg l^{-1} NAA and supplemented with sodium chloride (NaCl) at concentrations of (6 , 10, 14 ,22) ds m^{-1}.

Data recorded

The culture were incubated at the same conditions previously mentioned above.4 weeks later , the mor phological parameters were recorded as follows:
1-shoots numbers.
2- shoots length (cm).
3-vegetative fresh and dry weight (g).
the physiological parameters were recorded as follows:
1-Sodium ion concentration (mg g^{-1} dry weight).
2-calcium ion concentration (mg g^{-1} dry weight).
3-Potassium ion concentration (mg g^{-1} dry weight).

Ion Analysis

The vegetative shoots were washed with deionized distilled water for several times, then the shoots were dried in the microwave and until the weight was stable (for 5 minutes), 150 mg of dry shoots were then taken and placed in a glass beaker of 50 ml capacity and 9 ml of the digestion mixture was added to it. of nitric acid, perchloric acid and sulfuric acid in a ratio of 10: 4: 1, respectively, the pots were placed in a sand bath at a temperature of 80 ° C until the digestion was complete, and the digested material was dissolved for each treatment to 50 ml by adding deionized water. Calcium, potassium and sodium concen trations were estimated using a flame photometer model PFP7 of the British JENWAY company, while magnesium was determined using atomic absorption device A.A.S model NOVA 400 of the German company Analytikjena**.**

Experimental layout and Statistical Analysis:

The experiment designed as factorial experiment based on Completely Randomized Design (C.R.D), the factors included sodium azide and salt levels with 4 replications for each treatment. DATA analyzed by Genstat program version 12 Edition 12^{ed} and means were separated at 5% of probability using Duncan's test.

RESULTS

Effect of salinity levels and mutagenicity with sodium azide.

average shoots number

The results in Table (1) show the superiority of 6 dS m^{-1} Sodium chloride in giving the highest average number of shoots reached 5.92 shoots while while the lowest shoots number reached 1.33 shoots in 22 dS m^{-1} NaCl. the treatments of 10 and 14 dS m^{-1} of NaCl did not differ significantly between them, as for the effect of sodium azide ,It caused a significant decrease in the average number of shoots compared to the control treatment, and for interaction between salinity and NaN_3 , it was noticed that there was a decrease in the number of shoots upon mutatation with NaN_3 at both concentrations and at all levels of salinity ,the control treatment superior in gave the highest number of shoots reached 9.25 shoots, while the lowest shoots number reached 0.75 shoots in 0.5 mM of sodium azide with 22 dS m^{-1} of sodium chloride (Fig. 1)

Table 1:.Effect of salinity and NaN_3 levels on shoots number of strawberry cv. Albion after 4 weeks from cultured on MS medium.

NaCl level (dS m^{-1})	Concentrations of NaN3 mM			
	0	0.25	0.5	mean
6	9.25 a	4.00 bc	4.500 b	5.92 A
10	2.00 de	2.00 de	3.75 bc	2.58 B
14	3.75 bc	2.75 cd	1.25 de	2.58 B
22	1.500 de	1.75 de	0.75 e	1.33 C
mean	4.13 A	2.63 B	2.56 B	

Means in the same column or their interactions followed by the same letters are not significantly different (P<0.05), according to Duncan's test.

average length of shoots

The result shown in Table (2) that 6 dS m-1 NaCl was superior in giving the highest average length of shoots reached 2.47 cm. As for the effect of excess sodium azide on the average length of shoots, no significant differences between the treatments. For the interaction between salinity and NaN3 concentrations, 6 dS m-1 NaCl giving the highest average length of shoots reached 3.04 cm, while the interaction between the salt level of 14 ds m-1 with 0.5 mM of NaN3 gave the lowest shoot length reached 0.69 cm.

Table 2:. Effect of salinity and NaN_3 levels on shoots length (cm)of strawberry cv. Albion after 4 weeks from cultured on MS medium

NaCl level (dS m^{-1})	**Concentrations of NaN3 mM**			
	0	**0.25**	**0.5**	**mean**
6	3.04 a	2.23 abc	2.14 abcd	2.47 A
10	1.23 bcd	2.42 ab	1.46 bcd	1.70 B
14	1.49 bcd	1.13 bcd	0.69 d	1.10 B
22	0.80 cd	1.56 bcd	0.93 cd	1.10 B
mean	1.64 A	1.84 A	1.31 A	

Means in the same column or their interactions followed by the same letters are not significantly different (P<0.05), according to Duncan's test.

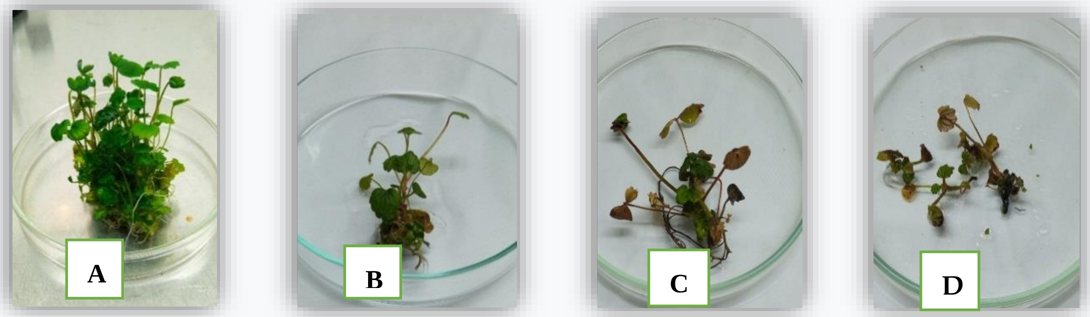

Figure1. Effect of Sodium azide and salt stress, 8 weeks after the treatment on *in vitro* Strawberry shoot cv. Albion. A- 6 dS m^{-1} NaCl B-0.25 mM NaN_3 interaction with 10 dS m^{-1} NaCl C- 0.5 mM NaN_3 interaction with 10 Ds m^{-1} D- 0.25 Mm NaN_3 interaction with 22 dS m^{-1}.

average of fresh weight (g)

The results (Table 3) showed revealed that 6 ds m^{-1} NaCl gave the highest average of fresh weight,reached 1,990 g, which was significantly superior to the rest of the treatments, while 22 dS m^{-1} NaCl gave the lowest fresh weight 0.344 g, Regarding to sodium azide,that 6 ds m^{-1} NaCl gave the highest average of fresh weight reached 2.255 g, while the lowest fresh weight at 0.5 mM sodium azide reached 0.685 g. For the interaction between NaN_3 and NaCl , the results showed that 6 ds m^{-1} NaCl which was significantly superior as compared to the other treatments reached 3.197 gm fresh weight,while 22 dS m^{-1} interaction with 0.25 mM sodium azide gave the lowest fresh weight reached 0.120 gm.

Table 3:.Effect of salinity and NaN3 levels on shoots fresh weight (gm) of strawberry cv. Albion after 4 weeks from cultured on MS medium

NaCl level (dS m^{-1})	Concentrations of NaN3 mM			
	0	**0.25**	**0.5**	**mean**
6	3.197 a	2.267 c	0.507 ghj	1.990 A
10	2.750 b	1.137 e	0.427 h	1.438 C
14	2.730 b	0.780 f	1.237 d	1.582 B
22	0.343 i	0.120 j	0.570 g	0.344 D
mean	2.255 A	1,076 B	0.685C	

Means in the same column or their interactions followed by the same letters are not significantly different (P<0.05), according to Duncan's test.

average of dry weight (g)

The results (Table 4) showed the superiority of 6 ds m^{-1} in giving the highest dry weight, reached 0.180 g, while 22 dS m^{-1} NaCl gave the lowest dry weight reached 0.052 g, Regarding to sodium azide effect, the control treatment was superior in giving the highest dry weight, reached 0.176 g, while 0.25 mM gave the lowest dry weight reached 0.010 g, Concerning to the interaction between NaCl and the mutagenic sodium azide, that 10 dS m^{-1} NaCl without mutagenic gave the highest dry weight reached 0.273 g in both treatments, while 22 dS m^{-1} of sodium chloride with 0.25 mM of sodium azide gave a minimum dry weight 0.033 g**.**

Table 4:. Effect of salinity and NaN_3 levels on shoots dry weight (gm) of strawberry cv. Albion after 4 weeks from cultured on MS medium

NaCl level (dS m^{-1})	Concentrations of NaN3 mM			
	0	**0.25**	**0.5**	**mean**
6	0.273 a	0.210 b	0.057 e	0.180 A
10	0. 273 a	0.102 cd	0.057 e	0.144 B
14	0.120 c	0.053 e	0.107 cd	0.093 C
22	0.035 e	0.033 e	0.087 d	0.052 D
mean	0.176 A	0.010 B	0.076 C	

Means in the same column or their interactions followed by the same letters are not significantly different (P<0.05), according to Duncan's test.

Sodium ion concentration (mg g^{-1} dry weight)

The results showed in Table (5) that the superiority of 14 dS m^{-1} sodium chloride in giving the highest concentration of the ion reached (32.04 mg sodium g^{-1} dry weight of the shoots), while the control treatment gave the lowest concentration reached (22.40 mg sodium gm^{-1}dry weight of shoots), and showed that the concentration of sodium ion increased with the increase of the concentration of the chemical mutagen, The interactions between NaCl and NaN_3 had a significant effect on the sodium ion concentration, reached the highest concentration (42.90 mg sodium g^{-1} dry weight of the shoots) when treated with 0.5 mM sodium azide interaction with NaCl 10 g l^{-1}, while control treatment giving the lowest concentration of sodium ion reached (11.93 mg sodium g^{-1} dry weight of the shoots).

Table 5:. Effect of salinity and NaN_3 levels on Sodium ion concentration (mg g^{-1} dry weight) of strawberry cv. Albion after 4 weeks from cultured on MS medium

NaCl level (dS m^{-1})	Concentrations of NaN3 mM			
	0	**0.25**	**0.5**	**mean**
6	11.93 h	31.95 cd	23.32 f	22.40 C
10	23.94 f	34.34 c	27.08 e	28.45 B
14	24.69 ef	31.89 cd	39.54 b	32.04 A
22	30.16 d	15.22 g	42.90 a	29.42 B
mean	22.68 C	28.35 B	33.21 A	

Means in the same column or their interactions followed by the same letters are not significantly different (P<0.05), according to Duncan's test.

Calcium ion concentration (mg g^{-1} dry weight)

The results shown in Table (6) revealed that (6 and 10) dS m^{-1} NaCl gave the highest concentration of calcium reached (5.968 and 5.988 mg calcium g^{-1}dry weight of shoots) respectively, and the two treatments were significantly superior to the rest of the treatments while 22 dS m^{-1} of NaCl gave the lowest concentration reached (4.910 mg of calcium gm^{-1} of the dry weight of the shoots), while 0.25 mM of sodium azide gave the highest concentration of the ion reached (6.019 mg calcium g^{-1} dry weight of shoots), and the control treatment gave the lowest concentration of ion reached (5.191

mg calcium g^{-1} dry weight of shoots) and did not differ significantly from the treatment of 0.5 mM sodium azide, which gave (5.333 mg calcium g^{-1} dry weight of shoots) with regard to the interaction between NaCl and sodium azide, the concentration of 0.25 mM of sodium azide and 6 ds m^{-1} was superior in giving the highest concentration of calcium ion reached (6.707 mg calcium g^{-1} dry weight of the shoots), while 0.5 mM of sodium azide interaction with 22 ds m^{-1} of sodium chloride had the lowest ion concentration reached (4.357 mg calcium gm^{-1} dry weight of shoots).

Table 6:. Effect of salinity and NaN_3 levels on calcium ion concentration (mg g^{-1} dry weight) of strawberry cv. Albion after 4 weeks from cultured on MS medium

NaCl Level (dS m^{-1})	Concentrations of NaN3 mM			
	0	**0.25**	**0.5**	**mean**
6	5.607 c	6.707 a	5.590 c	5.968 A
10	5.557 c	6.120 b	6.287 b	5.988 A
14	5.310 cd	5.167 d	5.097 d	5.191 B
22	4.290 e	6.083 b	4.357 e	4.910 C
mean	5.191 B	6.019 A	5.333 B	

Means in the same column or their interactions followed by the same letters are not significantly different ($P<0.05$), according to Duncan's test.

Potassium ion concentration (mg g^{-1} dry weight

The results in Table (7) that the superiority (6 and 10) dS m^{-1} NaCl in giving the highest concentration of ion (26.31 and 26.88 mg potassium g^{-1} dry weight of shoots) respectively, while the lowest concentration of Potassium ion at 22 dS m^{-1} NaCl (14.62 mg potassium gm^{-1} dry weight of shoots), and as for the chemical mutagenic effects of sodium azide, it was significantly superior to 0.25 mM sodium azide and gave the highest potassium ion concentration reached (29.46 mg potassium g^{-1} dry weight of shoots) and the treament was significantly superior to the rest of the treatments and the control treatment gave the lowest concentration of the ion reached (13.82 mg potassium g^{-1} dry weight of the shoots), and regarding the interaction between NaCl and NaN_3 ,sodium azide was superior at the concentration 0.25 mM interaction with 6 ds m^{-1} NaCl reached (33.06 mg potassium g^{-1} dry weight of shoots), while 0.5 mM sodium azide interation with 22 ds m^{-1} NaCl had the lowest concentration (8.29 mg potassium g^{-1} dry weight of the shoots).

Table 7:.Effect of salinity and NaN_3 levels on potassium ion concentration (mg g^{-1} dry weight) of strawberry cv. Albion after 4 weeks from cultured on MS medium

NaCl level (dS m^{-1})	Concentrations of NaN3 mM			
	0	**0.25**	**0.5**	**mean**
6	21.05 ef	33.06 a	24.83 d	26.31 A
10	19.75 f	31.73 ab	29.17 c	26.88 A
14	9.31 g	22.67 d	31.50 abc	21.16 B
22	5.18 h	30.39 bc	8.29 g	14.62 C
mean	13.82 C	29.46 A	23.45 B	

Means in the same column or their interactions followed by the same letters are not significantly different ($P<0.05$), according to Duncan's test.

DISCUSSION

Salinity stress is generally defined as the presence of excessive amounts of soluble salt that negatively affect the functions needed for normal plant growth and development. Salt stress is comprised of two harmful effects: osmotic stress leading to reduced water uptake, and ionic stress caused by the toxicity of specific ions (mainly Na^+ and Cl^-). Ionic stress leads to unrestrained overproduction of ROS (reactive oxygen species), such as superoxide radicals (O2·), hydrogen peroxide (H2O2), and hydroxyl radicals ($OH^{\cdot-}$). These reactive molecules accumulate to toxic levels and trigger oxidative damage in the cells and organelles by destroying membranes, proteins, enzymes, and nucleic acids ([14, 15, 16, 17]).

chemical mutagens are useful tools in crop improvement and have been used to produce abiotic stress tolerance and disease resistance in various plant crops, improving their yield [18] In the present study while *in vitro* exposure to sodium azide decreased in the number of shoots ,fresh and dry weight due to Phenotypically, the rapid effects of sodium azide on the meristematic cells in the cultivated explant is to inhibit cell multiplication due to its obstruction of the multiplication process in the S phase (S-phased) during the cell cycle in addition to some biochemical reasons, as it causes inhibition of the respiratory chain and transport chain Electron [19]. The mutagenic ability of the sodium azide mutagen depends on its production of an organic metabolic compound called B-azidoalanine, which stimulates chromosomal aberrations at a lower rate than other mutagens [20], which cause disruptions in growth, metabolic activity, protein inhibition, and DNA replication [11] The mutagen change the balance between growth stimulators and inhibitors [21]. The results of this study are consistent with the findings of [22] of a decrease in vegetative growth when plants were subjected to chemical mutagenicity.

CONCLUSIONS

The results showed that salinity caused an increase in sodium ion, while it caused a decrease in the co-centration of calcium and potassium ions, and this may be due to the fact that the increased absorption of sodium and chlorine as a result of adding them to the food medium has reduced the absorption of other nutrients [23] because it interferes and competes absorbed by the plant [24] found that the use of different mutants such as sodium azide reverses and reduces these negative effects and increases potassium and calcium ion absorption in plant tissues.

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