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Effect of Sodium Azide on Some Vegetative and Biochemical Properties of Strawberry under Polyethylene Glycol of Albion Variety *in vitro*

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ABSTRACT

In vitro experiment was carried out in the tissue culture laboratory of the Department of Horticulture and Landscaping, University of Anbar. This study showed the effect of four concentrations of the chemical stimulator sodium azide (SA) (0, 1, 2, 3 mmol) under four levels of water stress polyethylene glycol (PEG), that included (0, 0.6 and 0.9 and 1.2%). This study included phenotypic traits such as number of leaves, number of branching, plant height, the concentration of proline, catalase and hydrogen peroxide of strawberry plants *in vitro*. A factorial experiment was carried out using the CRD design with five replicates. The results showed that the concentration of 2 mmol of sodium azide was superior for the number of leaves (14.00 leaves/planlet), the number of branches (3.92 branches/plantlet) and the height of the plant (1.61 cm). However, the concentration of 3 mmol of sodium azide chemical mutagen gave 2.56 mg/g of proline, 0.69 µmol 100/mg d.w. of hydrogen peroxide and 19.91 AU/min/g of catalase enzyme. Moreover, the level of 0.6% polyethylene glycol improved the number of leaves and branches (12.00 leaves/planlet and 3.42 branches/plantlet). The concentration of 0.9% of polyethylene glycol increased proline by 2.43 mg/g and catalase enzyme by 19.59 AU/min/g.

Key words: Strawberry, sodium azide, polyethylene glycol, *in vitro*, chemical mutation

INTRODUCTION

The strawberry (*Fragaria ananassa* Duch) is one of the most significant micro fruits belonging to the Rosaceae family. It is exploited in human nutrition due to its elemental component and desirable taste. The world's consumption of strawberry fruits is increased as these fruits have anti-bacterial, anti-fungal and antioxidant features. This plant may contain phytochemicals like carotenoids, phenols and flavonoids which reduce cancer diseases (Padmanabhan *et al.*, 2016).

Water stress is one of the environmental factors affecting plant growth and productivity that decreases water availability and induces a number of changes in plant organs. Gas exchange in the leaves is limited, which reduces carbon metabolism and causes changes in the distribution of the products of carbon metabolism. Also, the differences in genetic material may cause a difference to withstand water stress. The decrease in leaf area in strawberry Elsanta, Elkat and Salut cultivars, stopped the roots in the Elkat cultivar. Elsanta cultivar gave the highest yield under water stress. Environmental stresses affect these fruits' growth negatively, especially water stress (Mozafari *et al.*, 2019). Water stress caused by polyethylene glycol is one of the most important non-biotic stimulating stresses, which indicates insufficient moisture required for plant growth and development (Riasat *et al.*, 2020). Some plants might show responses to different molecular, chemical and physiological factors to deal with these environmental changes under stress (Yosefi and Javadi, 2020).

Gomez *et al.* (2019) found that treating pineapple plants with the chemical mutagen sodium azide at concentrations (0, 0.15, 0.30 and 0.45 mmol) decreased the doubling rate and fresh weight of soluble phenols, while carotenoids increased and lipid oxidation products did not increase significantly. Iqbal *et al.* (2019) noticed the addition of sodium azide to the callus of the black seed plant led to an increase in antioxidants and inhibition of DNA damage. Therefore, the concentration of 100 mmol of sodium azide can increase the effectiveness of the catalase of the black seed plant and increase phenolic compounds, as well as lead to an increase in secondary metabolic compounds. The environmental result of the addition of mutants such as sodium azide is the increase of secondary metabolites in grapes such as flavinolol, flavonols, and anthocyanins in the callus (Cetin and Daler, 2021; Mahood, 2021). The characteristics of some plants, such as height, feather thickness, number of leaves, root length and thickness, and stem number, may vary when treating grape plants with sodium azide mutagenic at a concentration of 0.02 mg/l. The reason may be attributed to the effect of the mutagen on some molecular characteristics, which was shown by ISSR analysis (Rayan *et al.*, 2014). *In vivo* variation when using mutants such as sodium azide and guanidine hydrochloride; sodium azide reduced the vegetative characteristics except for the number of branches, leaves and leaf length, which increased the percentage of genetic variance in sodium azide was 57.71%. Jagtap *et al.* (2019) concluded that the necessity of using sodium azide to induce mutagenesis in the jasmine plant caused genetic variation through which it could tolerate abiotic stresses if the *ex vivo* treatment of jasmine plants led to an increase in callus size when adding 2 mg of sodium azide.

Water deficiency also might reduce plant growth, chlorophyll content, gas exchange and relative water content in leaves. Still, it increases dissolved solids, anthocyanins and proline, and an increase in anthocyanins and proline is one of the protective mechanisms for carrying water stress (Yenni *et al.*, 2022). Other researchers observed that exposing the fig plantlets of Sabz and Siah cultivars to several levels of polyethylene glycol (0, 2, 4 and 6%) led to a decrease in vegetative growth characteristics such as length, dry and fresh weight of shoots, while leaf area was not affected as well. The content of the two cultivars of proline differed, where it was higher in Sabz cultivar than in Siah (81.8 µmol/g). The soluble sugar content was also increased, and starch decreased. Kim *et al.* (2015) also found that exposing strawberry plants to different levels of polyethylene glycol led to the production of plants with shorter lengths and less weight. The survival rate improved after four weeks, and the level of 5 g/l of polyethylene glycol was more suitable for the growth of plants under water stress. Some biochemical characteristics such as peroxidase, superoxide dismutase, malondialdehyde, hydrogenreroxide and proline content may be affected. These components were increased in strawberry plants grown in ex vivo, exposed to water stress, and stimulated by polyethylene glycol (Yosefi and Javadi, 2020). Several levels of polyethylene glycol led to a decrease in dry weight at high concentrations, as well as a decrease in the doubling rate and height. The reason may be due to the cessation of water metabolism according to the genetic material of the strawberry.

Therefore, the aim of this study is to know the effect of the chemical mutagenic sodium azide on some morphological and biochemical properties of the Albion strawberry plant and to compare its behaviour under different levels of *ex vivo* water stress.

MATERIALS AND METHODS

The experiment was carried out in the tissue culture laboratory of the College of Agriculture, University of Anbar for the season 2020-21. The Albion transplantings were brought from Halabja, Sulaymaniyah. The purlins (top of approximately 1 cm long) were separated and transported to the laboratory (Khairallah and Ahmed, 2014). Then, it was cleaned well with liquid soap. After that, it was washed with tap water for an hour. Then moved to the laminar airflow cabinet for removing the infestations. The metallic and glass, including surgical blades, tongs, petri dishes, beakers and flasks, were disinfected after being washed with water, cleaner and distilled water. These tools were kept in the oven at 200°C for 2 h (Sekhi *et al.*, 2021). At the same time, the distilled water was sterilized in the autoclave at a temperature of 121°C and a pressure of 1.04 kg/cm^2 for 15 min. The explants were superficially sterilized by immersion in Clorox minor solution consisting of 6% sodium hypochlorite/wv. After that, the solution was diluted to 3% /wv by adding a few drops of Tween 20 diffuser to decrease surface tension. The plant parts were then washed with water thrice for 5 min, and then the plant parts were shifted to sterilized petri dishes to excise 0.5 cm runner tip and separate it using sterile scalpels and tweezers.

Standard MS media supported by (Caisson Company) was applied. Then, the media was enhanced by 30 g/l of sucrose, and growth regulators were applied depending on the requirements of the experiment (as detailed below). The pH was adjusted to 5.7-5.8 using NaOH or HCl, added 7.0 g/l of agar, filled with the volume to 1 liter, and melted using a hot plate magnetic stirrer until boiling. The prepared medium was distributed in glass vials with an amount of 10 ml in each vial, and the bottles were covered with their special caps after sterilizing by steam at a pressure of 1.04 kg/cm^2 and at 121°C for 20 min.

The growing tip of the Albion cultivar under study was cultivated on media equipped with a 0.5 mg/l BA added to it with 0.1 mg/l IBA. It was incubated at 24±1°C. It was kept for 16 h under the illumination intensity of 1000 lux with 8 h dark for four weeks. The resulting branches from the previous stage (growth stage) were transmitted to the multiplication media, which was equipped with 1.0 mg/l BA added to it 0.1 mg/l IBA, under the same conditions referred to in the previous paragraph (Kadhim *et al.*, 2020; Kadhim and Abdulhussein, 2021).

The solution prepared. Three mutagenic concentrations of SA, sodium azide were prepared (1, 2 and 3 mmol). The solutions of mutagen were located in sterilized flasks. A sterilizer disinfected the solutions at a pressure of 1.04 kg/cm^2 and 121°C for 20 min. The plants were completely soaked in the solution of each concentration for a full hour inside the stratified air table. Then, it was extracted and washed with sterile distilled water for 10 min with 3-5 folds shakings. After that, they were cultured on a growth medium (MS) with the same concentrations shown in the stage of plantlet growth for a whole month. Cultural media were prepared by the same concentrations as illustrated in the practice of plant growth. Then, three levels of water stress added were 0.6, 0.9 and 1.2%. The plantlets were cultured on the cultural media and left to grow for four weeks. The culture was repeated for another four weeks in the same media to complete the two-month period. This trial was laid out with five replicates. The growth data were recorded as each plantlet was considered an experimental unit.

The data were recorded for the number of leaves, the number of branches, plant height and concentration of proline, hydrogen peroxide, and catalase enzyme. A factorial experiment system was conducted using a completely randomized design. Then, the data were statistically analyzed by adopting the least significant difference test at a probability level of 5% following the electronic statistical program Genstat version (12).

RESULTS AND DISCUSSION

There were significant differences between the concentrations of the addition of the mutagen affecting the average number of strawberry leaves (Table 1). The level of the mutagen 2 m mol gave the highest average number of leaves reaching (14.00 leaves/ planlet). This did not differ significantly from the comparison treatment (15.00 leaves/ planlet), followed by level 1 mmol (10.75 leaves/ planlet). Contrarily, level 3 gave the lowest average number of leaves amounting to 6.67 leaves/planlet. Stress stimulator (PEG) among them had average number of leaves. The comparison treatment gave the highest average number of leaves, which was 14.00 leaves/planlet, then the concentration of 0.6% (12.00 leaves/planlet), and the concentration of 1.2 (10.25 leaves/planlet), while the concentration gave 0.9, the lowest average number of leaves was (10.17 leaves/planlet). There were significant differences between the combinations of the two factors. The comparison treatment gave the highest average number of leaves (18.00 leaves/ planlet). While the combination of 0.6% x 0 of the mutagen (SA) (15.67 leaves/planlet) and the combination of 2 mmol SA x 0 PEG (14.33 leaves/planlet). The three mmol x 0.9% overlap

Table 1. Effect of sodium azide (SA) and polyethylene glycol (PEG) on leaf number in strawberry cv. Albion

PEG \ S A	SA_0 (0.00 mM)	SA_1 (1.00 mM)	SA_2 (2.00 mM)	SA_3 (3.00 mM)	Mean
PEG_0 (0)	18.00	13.33	14.33	10.33	14.00
PEG_1 (0.6%)	15.67	9.67	14.00	8.67	12.00
PEG_2 (0.9%)	13.33	10.33	14.00	3.00	10.17
PEG_3 (1.2%)	13.00	9.67	13.67	4.67	10.25
Mean	15.00	10.75	14.00	6.67	
LSD (P=0.05)	SA	PEG		SA × PEG	
	1.71	1.71		3.42	

gave the lowest average number of leaves, which was three leaves/planlet.

There were significant differences between the concentrations of the addition of the mutagen by the effecting on the average number of branches of strawberry (Table 2). The level of the mutagen two mmol gave the highest average number of branches reaching 3.92 branches/plantlet, which did not differ significantly from the control treatment (3.83 branches/plantlet), followed by level 1 mmol (3.50 branches/plantlet). Contrarily, level 3 mmol gave the lowest average number of branches reaching 2.33 branches/plantlet. Stress stimulus (PEG) gave average number of branches. Furthermore, the comparison of treatments gave the highest average number of branches, which amounted to 4.17 branches/plantlet. The concentration 0.6% had 3.42 branches/plantlet, followed by concentration 1.2 (3.08 branches/plantlet). The lowest average number of branches was 2.92 branches/plantlet in 0.9. The comparison treatment gave the highest average number of branches, which amounted to 5.33 branches/plantlet, followed by a combination of concentration of 2 mmol of mutagen (SA) x 0.6% PEG (4.67 branches/plantlet) and a combination of 1 mmol of SA x 0 PEG. (4.33 branches/plantlet). While the overlap of 3 mmol x 0.9% PEG gave the lowest mean number of leaves, which was 1.00 branches/ plantlet.

The comparison treatment gave the highest plant height of 1.63 cm, followed by a concentration of 2 mmol (1.61 cm), then a concentration of 1 mmol (1.11 cm; Table 3). The level was three mmol (1.02), while there were no significant differences between the levels of PEG. The interaction indicated that there were significant differences between the combinations. The comparison combination gave the highest plant height of 2.17 cm, followed by a combination of 2 mmol x 0.6% PEG and a combination of 2 mmol x 0 PEG (1.63 cm) for each of the two combinations. While the combination of 3 mmol x 1.2 PEG gave a minimum plant height of 0.83 cm.

The levels of the mutagen SA caused a significant increase in the concentration of proline for nitrate (Table 4). The concentration of 3 mmol gave the highest proline acid content (2.57 mg/g), with concentrations of 2 and 1 mmol (2.02 mg/g). In contrast, the control treatment had the lowest concentration of 1.53 mg/g proline. It was also noted from the same table that there were significant differences among the levels of PEG in proline content, as the level of 0.9% gave the highest proline of 2.43 mg/g, then the level of 0.6% (1.96 mg/g), followed by the control (1.93 mg/g). In comparison, the level of 1.2% gave the lowest proline (1.81 mg/g). It was found that there were significant differences between the interaction combinations in the concentration of proline. The interaction combination of 3

Table 2. Effect of sodium azide (SA) and polyethylene glycol (PEG) on number of branches in strawberry cv. Albion

PEG \ SA	SA_0 (0.00 mM)	SA_1 (1.00 mM)	SA_2 (2.00 mM)	SA_3 (3.00 mM)	Mean
PEG_0 (0)	5.33	4.33	3.67	3.33	4.17
PEG_1 (0.6%)	2.67	3.33	4.67	3.00	3.42
PEG_2 (0.9%)	3.67	3.33	3.67	1.00	2.92
PEG_3 (1.2%)	3.67	3.00	3.67	2.00	3.08
Mean	3.83	3.50	3.92	2.33	
LSD (P=0.05)	SA	PEG		SA × PEG	
	0.93	0.93		1.86	

Table 3. Effect of sodium azide (SA) and polyethylene glycol (PEG) on plant height in strawberry cv. Albion

PEG \ SA	SA_0 (0.00 mM)	SA_1 (1.00 mM)	SA_2 (2.00 mM)	SA_3 (3.00 mM)	Mean
PEG_0 (0)	2.17	0.90	1.63	1.03	1.43
PEG_1 (0.6%)	1.43	1.27	1.63	1.17	1.38
PEG_2 (0.9%)	1.37	0.87	1.57	1.03	1.21
PEG_3 (1.2%)	1.57	1.40	1.60	0.83	1.35
Mean	1.63	1.11	1.61	1.02	
LSD (P=0.05)	SA	PEG		SA × PEG	
	0.27	NS		0.55	

Table 4. Effect of sodium azide (SA) and polyethylene glycol (PEG) on proline in strawberry cv. Albion

SA / PEG	SA_0 (0.00 mM)	SA_1 (1.00 mM)	SA_2 (2.00 mM)	SA_3 (3.00 mM)	Mean
PEG_0 (0)	1.49	1.86	1.92	2.45	1.93
PEG_1 (0.6%)	1.73	1.87	1.43	2.81	1.96
PEG_2 (0.9%)	1.44	2.58	2.78	2.94	2.43
PEG_3 (1.2%)	1.47	1.79	1.94	2.02	1.81
Mean	1.53	2.02	2.02	2.56	
LSD (P=0.05)	SA	PEG		SA × PEG	
	0.14	0.14		0.29	

mmol of mutagen x 0.9% PEG gave the highest concentration of 2.94 mg/g followed by an interaction between 3 mmol x 0.6% (2.81 mg/g). In comparison, the interaction gave two mmol of mutagenic x 0.06% of PEG, a minimum concentration of 1.43 mg/g.

The 3 mmol gave the highest concentration of hydrogen peroxide of 0.69 μmol 100/mg d.w. then the concentration was 2 mmol (0.64 69 μmol 100/mg d.w.) and the concentration had 1 mmol (0.59 69 μmol 100/mg/d.w.). However, the comparison treatment gave the lowest concentration (0.47 69 μmol 100 mg^{-1}d.w) of hydrogen peroxide. It was also observed from the table that there were no significant differences among the levels of PEG in hydrogen peroxide. From Table 5, it was found that there were significant differences among the interaction combinations in hydrogen peroxide. The interaction of 2 mmol of the mutagen x 0.9% of PEG gave the highest concentration of 0.81 69 μmol 100/mg d.w. followed by the interaction of 3 mmol x 0.6% (0.78 μmol 100/mg d.w.). While a comparison combination of mutagenic 0 x 0.9% PEG gave a minimum concentration of 0.42 69 μmol 100/mg d.w.

A concentration of 3 mmol gave the highest catalase enzyme concentration, reaching 19.91 AU/min/g (Table 6). While two mmol (19.01 AU/min/g), then the concentration was one mmol (18.29 AU/min/g). However, the comparison treatment gave a minimum concentration of 16.17 AU/min/g of catalase enzyme. It was also noted from the table that there were significant differences among the levels of PEG. The level of 0.9% gave the highest concentration of catalase, which amounted to 19.59 AU/min/g, then the level of 1.2% (18.01 AU/min/g), then the level of 0.6% (17.91 AU/min/g). In contrast, the comparison treatment gave the lowest concentration of catalase which was 17.90 AU/min/g. There were significant differences among interaction combinations in catalase. The interaction of 2 mmol of the mutagen x

Table 5. Effect of sodium azide (SA) and polyethylene glycol (PEG) on H2O2 in strawberry cv. Albion

SA / PEG	SA_0 (0.00 mM)	SA_1 (1.00 mM)	SA_2 (2.00 mM)	SA_3 (3.00 mM)	Mean
PEG_0 (0)	0.51	0.58	0.61	0.68	0.60
PEG_1 (0.6%)	0.51	0.56	0.56	0.78	0.60
PEG_2 (0.9%)	0.42	0.60	0.81	0.73	0.64
PEG_3 (1.2%)	0.45	0.62	0.58	0.55	0.55
Mean	0.47	0.59	0.64	0.69	
LSD (P=0.05)	SA	PEG		SA × PEG	
	0.08	NS		0.16	

Table 6. Effect of sodium azide (SA) and polyethylene glycol (PEG) on catalase enzyme in strawberry cv. Albion

SA / PEG	SA_0 (0.00 mM)	SA_1 (1.00 mM)	SA_2 (2.00 mM)	SA_3 (3.00 mM)	Mean
PEG_0 (0)	15.29	18.37	19.00	18.93	17.90
PEG_1 (0.6%)	16.67	18.53	16.00	20.43	17.91
PEG_2 (0.9%)	16.33	18.99	22.67	20.35	19.59
PEG_3 (1.2%)	16.40	17.27	18.43	19.93	18.01
Mean	16.17	18.29	19.03	19.91	
LSD (P=0.05)	SA	PEG		SA × PEG	
	0.35	0.35		0.69	

0.9% of PEG gave the highest concentration of 22.67 AU/min/g, followed by three mmol x 0.6% of PEG (20.43 AU/min/g). At the same time, interference 0 of the x 0 mutagen of PEG gave the lowest concentration of 15.29 AU/min/g.
It is noted from the above results that enhancing the nutrient medium with the chemical mutagen sodium azide at a concentration of 2 mmol led to the improvement of some vegetative characteristics, as shown in Tables 1 and 2, except for the plant height which decreased (Table 3). Further, increasing the levels of the mutagen to more than two mmol led to a reduction or reduction of these characteristics. The cause may be due to the fact that the mutagen may be effective in inducing the mechanisms of manufacturing secondary metabolic compounds such as phenols (Ctin and Daler, 2021) who noticed that fortifying the nutrient medium for grape lime production with the mutagen of sodium azide increased the formation of phenols, flavanols, flavonols and anthocyanins.
The decrease in these traits may be attributed to the fact that the mutagen caused a doubling of the genetic material. Still, it did not express itself, which led to an autosomal variation using this mutagen, which led to the variance in vegetative traits. This genetic variation was confirmed using ISSR analysis when studying the effect of sodium azide to induce genetic variation in grapes *ex vivo* (Rayan *et al.*, 2014). Similarly, Tawfik and Fathi (2020) noticed significant genetic differences in the plants of Cardinia, which were reflected in the content of chlorophyll, the length of the shoot, the number of lateral roots, the number of leaves, the number of branches, the length of the leaf, and the length of the root, as well as some physiological characteristics such as chlorophyll and carotene. Low concentrations of sodium azide may cause positive responses, and higher concentrations caused negative responses in jasmine callus (Jagtap *et al.*, 2019). Sodium azide may also participate in the synthesis of some compounds such as thymoquinone from the reduced formula by interfering with the compounds aldehydes, which by increasing may act as an anti-proliferative, which led to a decrease in growth and thus a decrease in vegetative characteristics (Iqbal *et al.,* 2019). Also, the presence of the azide ion inhibited the enzyme cytochrome oxidase and the process of oxidative phosphorylation, reduced chlorophyll, increased proline and other antioxidants, and increased the superoxide dismutase and proline as a result of the accumulation of O_2 and its transformation into hydrogen peroxide, which affected growth. Also, the low concentrations of sodium azide, which led to the improvement of some growth and biological traits, were due to the increase in antioxidant activity and the reduction of DNA damage *ex vivo*, and the stimulation of the production of metabolic compounds such as phenols, flavonoids and carotene (Iqbal *et al.*, 2021). The increase in growth characteristics to a certain extent was also attributed to the fact that sodium azide enhanced the protection of pigments and lipid oxidation, and some aldehyde compounds collected in certain proportions, which by increasing the levels of mutagens, these compounds increased, which inhibited the vegetative growth characteristics (Gomez *et al.*, 2019). It was noted that the chemical mutagen sodium azide was very efficient in the increase of proline and peroxidase enzyme catalase, which indicated that this chemical compound led to an increase in the antioxidant activity as it resulted in an increase in these biochemicals *ex vivo*. Mutagens led to stimulation mutations and abnormalities in the chromosome and other genetic mutations, thereby effecting some biochemical properties i.e. the activated plant metabolism resulting from the application of mutagens (Mostafa and Abou Alhamd, 2015). Furthermore, the mutagen led to an increase in the activity rate of the plant cell, which resulted in some biochemical properties, or the rearrangement of the genetic material, causing an increase in cell metabolism and its elongation reflecting in some biochemicals and physiological properties (Sekhi *et al.*, 2021). The mutagen also caused an imbalance in the metabolic activities, which resulted in the irregularity of the plant's resistance to stress, as it was possible to observe these effects under different stresses, such as water stress, which caused the accumulation of active oxygen species that increased the antioxidant compounds (Mostafa and Abou Alhamd, 2016).
The results also indicated that the increase in stress levels led to a decrease in growth characteristics and some vital characteristics (Tables 1, 2, 4, 5 and 6). The reason may be

attributed to the fact that water stress caused changes in light reactions, an increase in the rate of transpiration, and a change in the distribution of dry matter, which resulted in a change in growth characteristics resulting from inhibition. The process of carbon metabolism was an important factor in improving growth characteristics. These responses may vary according to the genetic material, as the genotypes that withstand water stress had high water use efficiency when water availability was low. Among these traits affected by stress are chlorophyll content, gas exchange, and relative water content in leaves, which decreased when water stressed while TDS increased, anthocyanin, and proline, as these were the mechanisms that explained the water stress tolerance of strawberry plants (Yenni *et al.*, 2022). In the same direction, the results of our research went with Islam *et al.* (2019), where it was important to note that the intensity of the doubling rate and the vigor of the plants decreased at high levels of PEG, as increasing the concentrations of PEG may cause the death of the cells of the plants. This inhibitory effect was a result of stopping cell elongation as a consequence of reduced bulging pressure due to reduced hydraulic conductivity in plant cells (Clapa and Harta, 2021).

Water stress may reduce the relative water content in the leaves, which affected the cell activity and also affected the physiological and phenotypic characteristics of strawberry plants, such as the dry weight of the roots and the shoot, as it may cause a decrease in leaf area and dry matter (Thokchom *et al.*, 2019). Growth stunting was the plant's first response to lack of water. Growth was achieved through cell expansion, division, and differentiation, which included complex interactions between physiological, molecular, and phenotypic characteristics. The type and quantity of plant growth depended on those processes, and these processes were affected by a lack of water. PEG may be used in tissue culture as a stimulus to obtain tensile strength. In addition, the negative effects of water stress may affect root dry matter and shoot under tissue culture conditions in strawberries (Mozafari *et al.*, 2018) and grapes (Mohammadzadeh *et al.*, 2018).

CONCLUSION

It was noticed that the vegetative and vital growth characteristics improved by increasing the concentration of the chemical mutagen, sodium azide (SA). The concentration improved by two mmol of the number of leaves, the number of branches, and the height of the plant. Also, the increase in water stress decreased the vegetative growth characteristics. The control treatment gave the highest number of leaves and number of branches of strawberry plants, and it increased the antioxidants' resistance to stress, as the level of 0.9% of polyethylene glycol (PEG) gave the highest concentration of proline and catalase.

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