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RESEARCH ARTICLE

Enhancement drought tolerance of sugar beet (*Beta vulgaris* L.) *in vitro* by sodium azide

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Abstract

Drought represents the most significant environmental challenge impacting the sugar beet growth and productivity. Sodium azide (NaN_3) is relatively safe to handle and the most efficient mutagen. So, the objective of the present investigation was to study the effect of presoaking two sugar beet cultivars seeds in various concentrations of sodium azide to improve drought tolerance *in vitro*. Results showed that the polyethylene glycol (PEG) at 5% or 10% caused water stress in medium and lead to highly significant reduction in shoot length, fresh weight, leaves number and photosynthetic pigment (Chl *a*, *b* and car). The 10% of PEG-6000 recorded the maximum reduction in growth parameter of two studied sugar beet cultivars. Otherwise, the tested sodium azide treatments improved sugar beet growth under stressed and unstressed plants. Presoaking of sugar beet seeds in 8 mM of sodium azide raises the shoot length, fresh weight, number of leaves and photosynthetic pigment of two cultivars and the most raising reported at unstressed plants. Proline content and antioxidant enzymes (POD, SOD and CAT) increased with increasing in poly ethylene glycol concentration. Moreover, sodium azide triggers the highest production of antioxidants enzymes in two cultivars of sugar beet under drought stress. Finally, presoaking of seeds in 4 or 8 mM of sodium azide can be enhancing drought tolerance in sugar beet.

Keywords: Sugar beet; Drought; Sodium azide; Stress tolerance; Antioxidant enzyme.

1. Introduction

Sugar beet (*Beta vulgaris* L.) is classified within the Chenopodiaceae family, which widely recognized as the most important sugar-producing crop globally, following sugarcane (Mubarak *et al.,* 2016). It contributes to nearly one-fifth of the world's sugar consumption, comprising approximately 40% of the total global sugar output. Additionally, sugar beet serves as a crucial source for ethanol production in the bioenergy sector and plays a significant role in fodder industries (Hosseini *et al.,* 2019; Subrahmanyeswari & Gantait, 2022). In Egypt, which is a semi-arid region, sugar beet is cultivated alongside sugarcane to meet the increasing demand for sugar consumption, which has risen alongside population growth. In Egypt, during the 2021/2022 season, the cultivated area for sugar beet was approximately 597.9 thousand feddans, resulting in a production of about 12.54 million tons (Ministry of Agriculture and Land Reclamation 2022).

Sugar beet exhibits a shorter productivity period and requires less water compared to sugarcane (Abdelaal *et al.,* 2015; Brar *et al.,* 2015). In sugar beet, sucrose comprises up to 18% of the plant's fresh weight, serving as the primary form of reduced carbon utilized in long-distance transport within most crop plants (Hermans *et al.,* 2005). Sugar beet is inherently challenging to propagate conventionally, due to its intractable nature and not flowered in Egypt because it needs to expose the roots to a low temperature 4-8 degrees

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Celsius, followed by a rise in temperatures, and these conditions are not available in Egypt (Romano, 2022). Consequently, various biotechnological techniques and tools focusing on *in vitro*-created comperhensive renovation coupled with genetic improvement are gaining popularity as effective alternatives to title the multifaceted difficulties associated with its traditional spread conventional propagation. (Subrahmanyeswari & Gantait, 2022). Extensive research achievements have been accomplished in recent years, focusing on optimizing *in vitro* protocols of *in vitro* for direct and callus-mediated regeneration, somatic hybridization, production of homozygous lines, and sugar beet of genetic transformation (Mubarak *et al.,* 2016; Taleghani, *et al.,* 2022).

Sugar beet production is affected by numerous factors, but drought considered a critical limitation factor, leading to yield reductions ranging from 5 to 30% and directly effect on development and growth of plant, production of pigment, and rate of photosynthetic (Hoffmann, 2010; Chołuj *et al.,* 2014). In addition to, Moosavi *et al.* (2017) who reported that drought stress exhibited a notable effect on sugar beet root yield, root dry weight and total dry weight as well as on leaf dry weight.

Using tissue culture technique has made it possible to develop the tolerance characters through the application of selective pressure in culture conditions (Sakhanokho & Kelley, 2009). Poly ethylene glycol (PEG) may help in developing an appropriate selection strategy by acting as a nonpenetrating osmotic agent that lowers the water potential of the medium (Biswas *et al.,* 2002). The growth parameters, biochemical and physiological characters of wheat were considerably influenced by PEG treatment (Hayoun *et al.,* 2023).

Sodium azide (NaN_3) was a widely recognized as a relatively safe, non-carcinogenic and highly efficient chemical mutagen (Salvi *et al.,* 2014; Dubey *et al.,* 2017). Moreover, it was commonly utilized to induce mutagenesis in *in vitro* plant systems (Türkoğlu *et al.,* 2022). Also, numerous studies have demonstrated the role and effectiveness of sodium azide to in enhance crop resistance against abiotic and biotic stresses (Olawuyi and Okoli, 2017; Hussain *et al.,* 2022). Hussain *et al.* (2022) reported that sodium azide treatments induced point mutations in the genome of plant, resulting in the production of proteins with different functions compared to non-mutant plants. Induced mutations had played an important role in developing various plant traits in different crop species, such as drought tolerance, disease resistance, adaptability, earliness, yield and other morphological characters (AL-Qurainy and Khan, 2009). Therefore, this study is aimed to investigate the effect of various sodium azide concentrations (4mM and 8mM) on two sugar beet cultivars to alleviate drought stress (by PEG) *in vitro*.

2. Materials and methods

2.1. Plant materials

The present investigation was conducted on two sugar beet cultivars namely Collins and Del 1135 R2, obtained from SCRI, ARC, Egypt. Its source from France.

2.2. Methods

The experiment was conducted in laboratory of tissue culture, Department of Agronomy, Fac. Agric., Suez Canal University, Ismailia Governorate, Egypt. Three concentrations of sodium azide (SA) (0 mM, 4 mM and 8 mM) were used as a mutagen to induce genetic variation in sugar beet cultivars. Sugar beet seeds were sterilized with 70% ethanol for 1min then soaking for 20min in a 25% solution of Clorex® containing NaOCl 5.25% with the addition of a tween drop, then washed with sterile distilled water 3-5 times. The seeds sterilized before were soaked in sodium azide (NaN_3) (0 mM, 4 mM and 8 mM) for 1hrs, finally washed with sterile distilled water 3-5 times.

The seeds were placed on medium of MS (Murashige and Skoog, 1962) appended with

sucrose 30 g/l, plant agar7 g/l) in vials. The medium pH was reached to 5.8 with 1N KOH and 1N HCL then sterilized in autoclaving at 121°C for 20 min. Four seeds were cultured into vial (240- ml baby food jar) containing 35 ml / vial cultured medium and incubated for four weeks under 25±2°C, 16 h/day light. Ten replicates for each cultivar were used in completely randomized design.

In vitro screening on media stressed with three different concentrations of polyethylene glycol (PEG) (0, 5 and 10 %) as a selection agent were attempted to develop drought-tolerant cultivars. Shoot fresh weight, shoot length and number of leaves of sugar beet plantlets as growth parameters were calculated after four weeks from sowing.

2.3. Biochemical determinations

2.3.1. Determination of photosynthetic pigments:

The plant pigments were spectrophotometrically determined in the fresh leaves according to Lichenthaler and Wellburn (1983). Chlorophyll a, b and carotenoids concentration were calculated as mg $100g^{-1}$ fresh weight (FW).

2.3.2. Determination of proline content:

Proline was determined using the following method was described by Bates *et al.* (1973). 0.5 g of fresh leaves was digested by aqueous 3% of sulphosalicylic acid (10 ml). For estimation, 2 mL of the extract was added glacial acetic acid (2 mL) and ninhydrin solution (2 mL). The blend underwent heating in a boiling bath for 1h. The reaction was stopped by tubed transferred to an cold bath. Incorporate 4ml of toluene into the mixture and stir thoroughly for twenty to thirty seconds. The layer of toluene was isolated and allowed to room temperature. The intensity of red color was measured at 520 nm relative to the blank of toluene. The contents of proline were determined using the standard curve of L-proline (Serva). The concentration of proline is expressed as mg per g of fresh weight, with a correction factor of 0.0285 applied.

2.3.3. Estimated activity of antioxidant enzyme

Enzymes extract was determined according to Urbanek *et al*. (1991).

2.3.4. Peroxidase activity (POD)

Peroxidase activity was assessed using O-dianisidine 0.1%and hydrogen peroxide 0.2 M, with measurements taken at 430 nm (Urbanek *et al*., 1991). POA was defined as the change of optical density 1.0 per milligram of fresh weight per minute. Mixture consisted of 2.5 ml of 0.1 M buffer phosphate (pH 6.5), 0.2 ml of 0.1% solution of O-dianisidine, 0.2 ml extract of enzyme and 0.2 ml of 0.2 M solution of hydrogen peroxide.

2.3.5. Catalase activity (CAT)

According to (Urbanek *et al.*, 1991), catalase was estimated by measured the H_2O_2 oxidation at 240 nm. CAT activity was defined as the amount of enzyme, which decomposes 1 mM H_2O_2 per mg^{-1} FW.minute. Reaction mixture consisted of 2.6 ml of 0.1 M buffer of phosphate (pH 6.8), enzyme extract 0.2 ml and hydrogen peroxide solution 0.2 ml of 0.2 M.

2.3.6. Superoxide dismutase activity (SOD)

Superoxide dismutase activity was estimated by ability measureed to inhibit nitro blue tetrazolium (NBT) reduction of at 560 nm as according with Beauchamp and Fridovich (1971). One unit of enzyme activity appears the amount of enzyme required for 50 % inhibit of NBT decrease. mixture contained 0.2 ml of enzyme extract, 0.25 ml for both of methionine (13 mM), NBT (80 μM) and EDTA (0.1mM). Then, made up the volume to 3.0 ml with buffer, at the end, added riboflavin (50 μM) 0.25 ml. shaken the the tubes and placed 30 cm away from light source. The reaction was allowed to run for twenty min. and the reaction was stopped by switching off the light.

2.4. Statistical analysis

Data was collect, checked, revised, and organized in figures and tables and were showed to outliers' revelation and handling. Data were presented as means and standard deviations. Differences between treatment groups were performed using

Multivariate difference analysis (MANOVA). ANOVA was followed by Duncan's Multiple Range Test (DMRT) due to compare between treatment groups at 0.05 levels. All statistical analyses were done using the Package of computer software Statistical for Social Science SPSS (IBM-SPSS ver. 26.0) (Knapp, 2017).

3. Results

Table 1 The fresh weight of shoots affected significantly by different tested concentrations of polyethylene glycol (0, 5, 10 %) and sodium azide (0, 4, 8 mM). Fresh weight showed in treatment groups (0,0), (0, 5%), (0, 10%), (4mM, 0), (4mM, 5%), (4mM, 10%), (8mM,0), (8mM, 5%), (8mM, 10%); in c.v. Collins showed an average (±SD) of 1.57±0.21, 0.64±0.12, 0.33±0.02, 2.06±0.18, 1.17±0.23, 0.86±0.12, 3.59±0.31, 2.15±0.19 and 1.50±0.27 g; respectively. The disparity between groups was found to be highly significant, as indicated by analysis of difference by one-way ($p<0.001$). Moreover, the shoot fresh weight in c.v. Del 1135 R2 were 1.07±0.08, 0.47±0.16, 0.35±0.05, 2.65±0.19,1.65±0.26, 0.72±0.18, 2.83±0.25, 2.42±0.18 and 1.93±0.08 g; respectively, and the difference between groups in c.v. Del 1135R2 was highly significant ($p<0.001$). Treatments by sodium azide significantly enhanced the shoot fresh weight at unstressed and stressed plants (Figure 1).

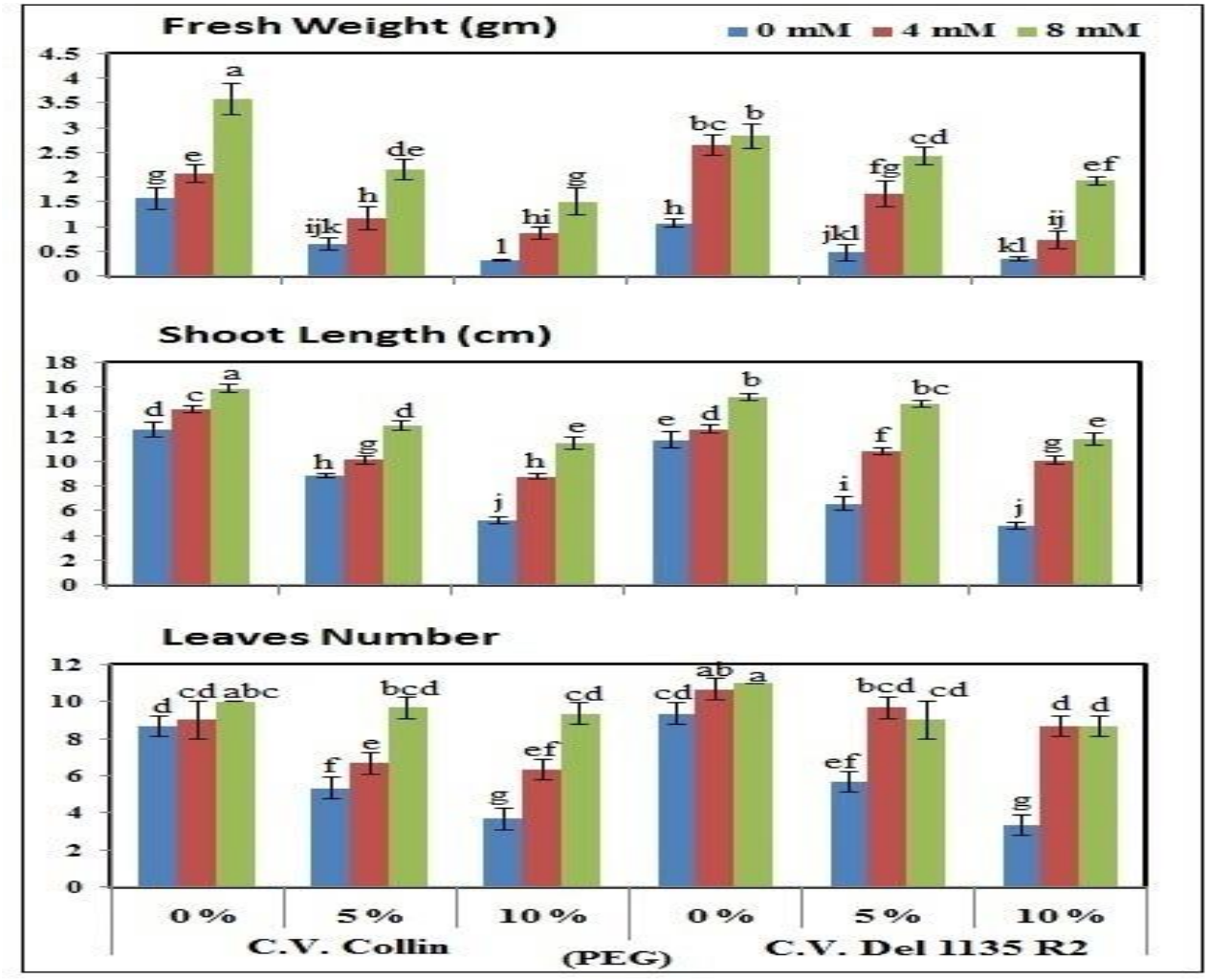

Figure 1. Shoot fresh weight, shoot length and leaves number of two sugar beet cultivars (Collins and Del 1135 R2) at different concentrations of sodium azide (0, 4mM, 8mM) and poly ethylene glycol (0, 5%, 10%). Bars followed by different letters are significantly different according to Duncan's Multiple Range Test (DMRT).

Shoot length (cm) in treatment groups in c.v. Collins showed an average (±SD) of 12.57±0.60, 8.83±0.21, 5.20±0.30, 14.23±0.25, 10.10±0.36, 8.77±0.25, 15.90±0.36, 12.90±0.36 and 11.50±0.50; The variation among groups was highly significant, respectively. However, shoot length (cm) in treatment groups in c.v. Del 1135 R2 showed an average (±SD) of 11.73±0.70, 6.53±0.55, 4.80±0.26, 12.60±0.36, 10.80±0.26, 10.07±0.31, 15.17±0.29, 14.67±0.29 and 11.77±0.47; The variance between groups was highly significant, respectively. In two cultivar, sodium azide raised shoot length significantly at normal and stress condition. The highest shoot length showed with 8 mM sodium azide at normal condition followed by 8 mM sodium azide at high osmotic stress (Figure 1&2).

Number of leaves in Collins showed an average (±SD) of 8.67±0.58, 5.33±0.58, 3.67±0.58, 9.00±1.00, 6.67±0.58, 6.33±0.58, 10.00±0.00, 9.67±0.58 and 9.33±0.58; respectively. However, in Del 1135 R2 showed an average (±SD) of 9.33±0.58, 5.67±0.58, 3.33±0.58, 10.67±0.58, 9.67±0.58, 8.67±0.58, 11.00±0.00, 9.00±1.00 and 8.67±0.58; The variances between groups in the 2 cultivars was highly significant, respectively (p<0.001). Leaves number results showed a significant elevation with sodium azide treatments and a significant reduction with osmotic stress in two cultivars (Figure 1&2).

Figure 2. Effect of different concentrations of sodium azide (0, 4mM, 8mM) on two sugar beet cultivars Collins (A) and Del 1135 R2 (B) at 10% poly ethylene glycol.

Chlorophyll a content observed in treatment groups; (0, 0), (0, 5%), (0,10%), (4mM, 0), (4mM, 5%), (4mM,10%), (8mM,0), (8mM, 5%), (8mM,10%), in Collins recorded an average (±SD) of 9.00±0.20, 7.94±0.07, 6.21±0.13, 16.90±0.31, 12.45±0.34, 10.25±1.27, 24.07±0.30, 23.07±0.55 and 20.51±0.55; The variances among groups was highly significant,

respectively. But, chlorophyll a in Del 1135 R2 showed an average (±SD) of 10.59±0.37, 6.92±0.15, 6.07±0.13, 19.95±0.31, 17.35±0.24, 14.92±0.15, 26.95±0.36, 25.44±0.77 and 21.06±0.20; the difference among groups was highly significant, respectively. Chlorophyll b contents in Collin reported an average (±SD) of 4.84±0.35, 3.90±0.29, 2.07±0.12, 7.28±0.26, 6.64±0.36, 6.56±0.33, 9.90±0.65, 8.64±0.36 and 8.30±0.24; the difference among groups was highly significant, respectively. On the other hand, chlorophyll b in Del 1135 R2 showed and average (±SD) of 4.890.18, 3.890.22, 2.050.11, 7.630.18, 7.140.39, 6.890.30, 10.970.08, 9.220.85 and 8.300.33; the difference between groups was highly significant, respectively (Figure 3). Osmotic stress caused a significantly reduction in chlorophyll content. On the contrary, sodium azide made significantly enhanced in chlorophyll content at all treatment groups.

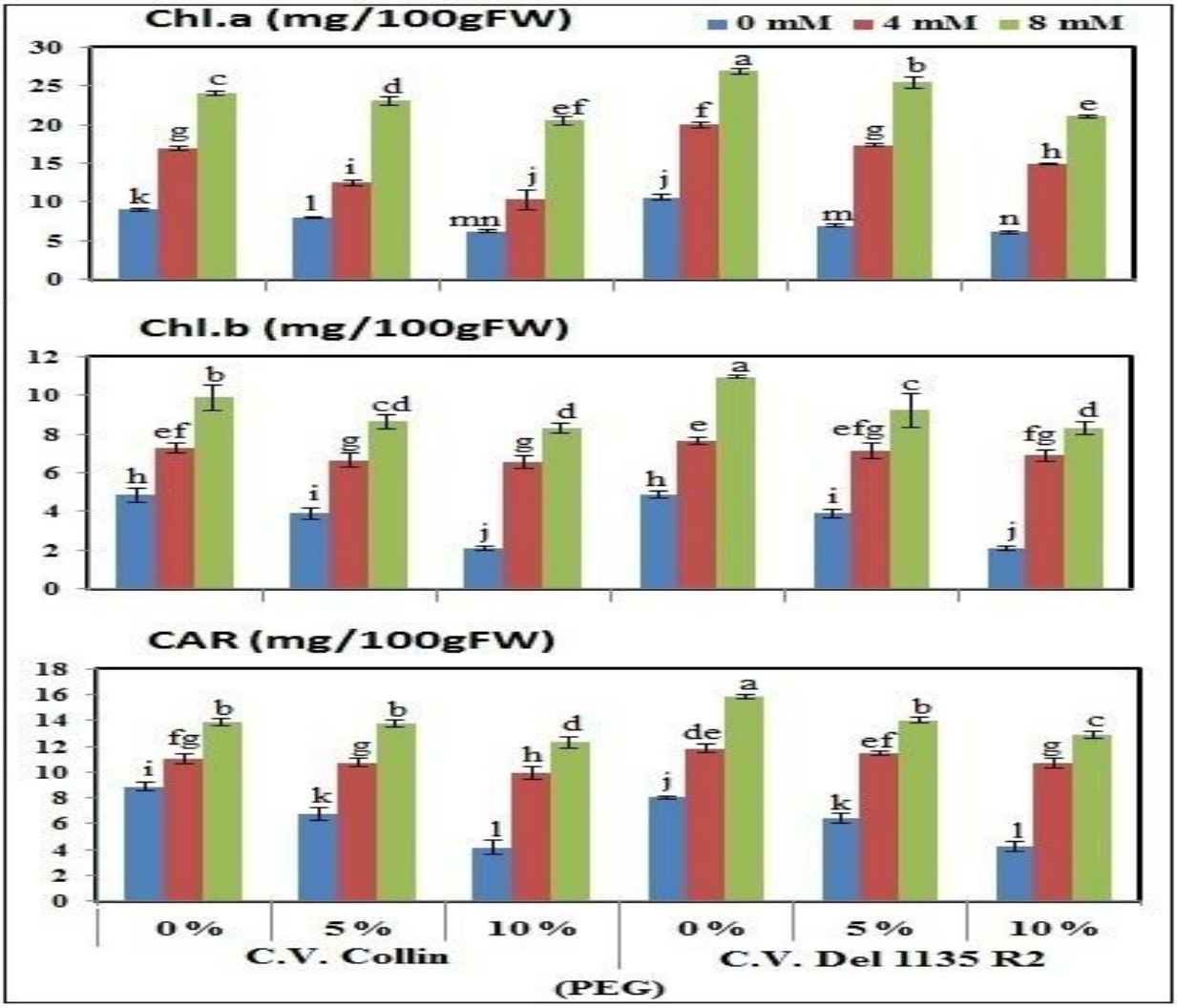

Figure 3. Chl a, Chl b and carotenoids content of two sugar beet cultivars (Collins and Del 1135 R2) at different concentrations of sodium azide (0, 4mM, 8mM) and poly ethylene glycol (0, 5%, 10%). Bars followed by different letters are significantly different according to Duncan's Multiple Range Test (DMRT).

Carotenoids content in c.v. Collins showed an average (±SD) of 8.87±0.30, 6.77±0.51, 4.15±0.56, 11.01±0.41, 10.71±0.33, 9.96±0.47, 13.85±0.25, 13.78±0.26 and 12.29±0.44; The variances between groups was highly significant, respectively, as revealed by one-way ($p<0.001$). However, in Dell showed an average (±SD) of 8.04±0.12, 6.40±0.40, 4.24±0.42, 11.84±0.29,

11.46±0.20, 10.70±0.37, 15.85±0.18, 14.02±0.19 and 12.89±0.28; respectively and the difference between groups in C.V. Del 1135 R2 was highly significant ($p<0.001$). The level of carotenoids in the two cultivar was a significant decreased with osmotic stress, while it was raised significantly with sodium azide treatment (Figure 3).

Average (±SD) of proline content in treatment groups; (0, 0), (0, 5%), (0,10%), (4mM, 0), (4mM, 5%), (4mM, 10%), (8mM, 0), (8mM, 5%), (8mM, 10%) in Collins were 6.01±0.14, 9.12±0.27, 10.61±0.79, 14.78±0.36, 15.19±0.19, 15.82±0.22, 17.99±0.22, 16.92±0.17 and 18.39±0.40; respectively (Figure 4). However, in Del 1135 R2 were 6.12±0.13, 7.62±0.36, 10.87±0.23, 12.28±0.09, 13.04±0.48, 14.18±0.44, 15.34±0.23, 16.53±0.35, 16.80±0.24; The variance between groups showed a notably high level of significance ($p<0.001$) in two cultivars, respectively. The treatment by polyethylene glycol and sodium azide were enhanced the proline content in two cultivar over than the control (0,0).

In two cultivars, the cellular antioxidant enzymes, (SOD), (POD), and (CAT), were supplemented with sodium azide treatment. (Figure 4).

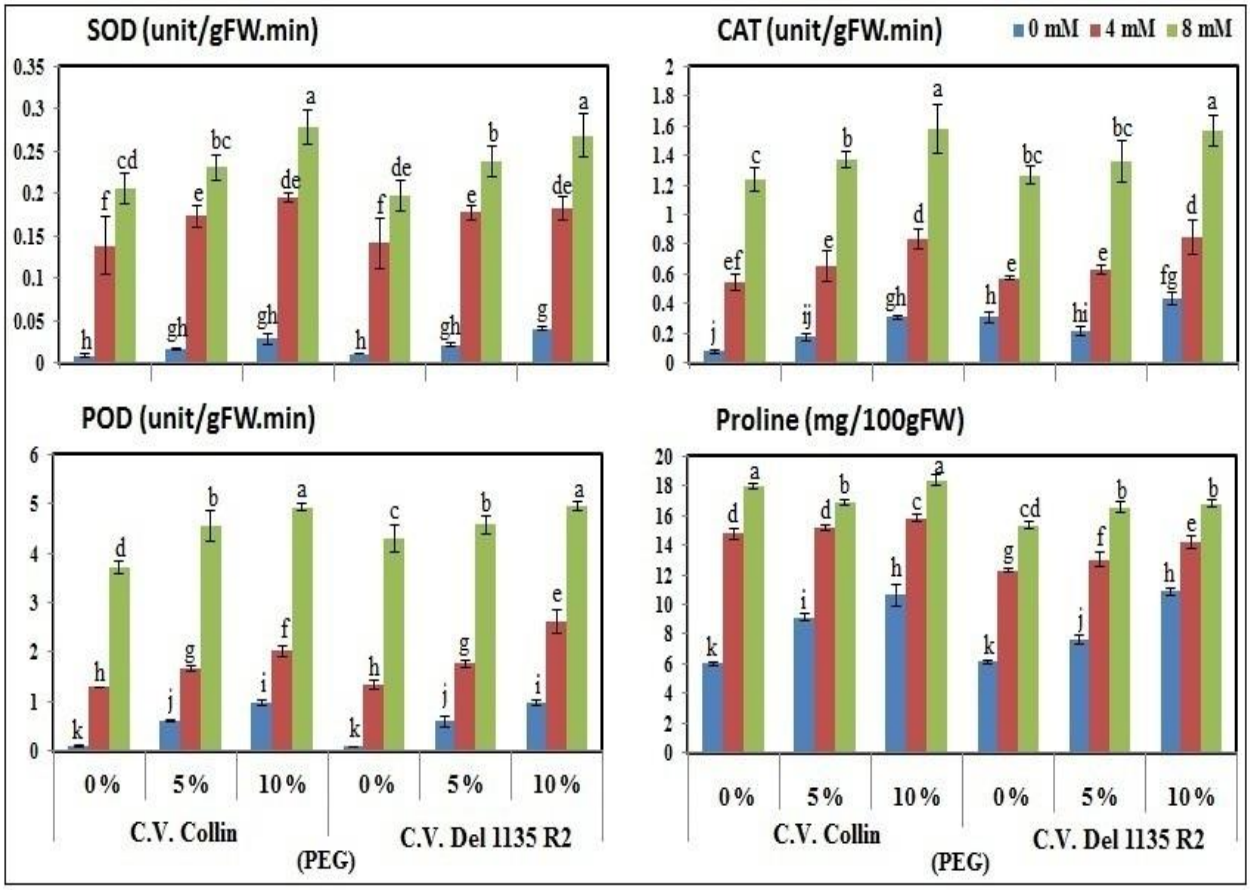

Figure 4. Antioxidant enzymes activity (SOD, CAT, POD) and proline content of two sugar beet cultivars (Collins and Del 1135 R2) at different concentrations of sodium azide (0, 4mM, 8mM) and poly ethylene glycol (0, 5%, 10%). Bars followed by different letters are significantly different according to Duncan's Multiple Range Test (DMRT).

In Collins, average (±SD) of SOD activity in treatment groups were 0.01±0.00, 0.02±0.00, 0.03±0.01, 0.14±0.03, 0.17±0.01, 0.19±0.01, 0.21±0.02, 0.23±0.01 and 0.28±0.02; respectively and the variances among groups was highly significant as revealed by one way ($p<0.001$). However, in Del 1135 R2 were 0.01±0.00, 0.02±0.00, 0.04±0.00, 0.14±0.03, 0.18±0.01, 0.18±0.01, 0.20±0.02, 0.24±0.02 and 0.27±0.03; respectively and the difference

between groups was highly significant. Moreover, POD activity in c.v. Collins showed an average (±SD) of 0.09±0.01, 0.07±0.02, 0.97±0.61, 1.28±0.02, 1.67±0.07, 2.01±0.10, 3.71±0.11, 4.55±0.33, 4.93±0.07; respectively. The difference between groups was highly significant ($p<0.001$). While, POD activity in Del 1135 R2 showed an average (±SD) of 0.09±0.00, 0.59±0.10, 0.97±0.05, 1.34±0.10, 1.76±0.07, 2.62±0.24, 4.29±0.27, 4.57±0.17 and 4.94±0.10; respectively, and the difference between groups was highly significant. In addition to, average (±SD) of CAT activity in Collin were 0.08±0.01, 0.17±0.03, 0.31±0.01, 0.54±0.06, 0.66±0.11, 0.84±0.07, 1.23±0.08, 1.37±0.06 and 1.58±0.16; respectively. The difference amongr groups was highly significant ($p<0.001$). However, in Del 1135 R2 were 0.31±0.03, 0.21±0.03, 0.43±0.04, 0.57±0.02, 0.63±0.03, 0.84±0.11, 1.26±0.06, 1.36±0.14 and 1.56±0.10; respectively, and the difference between groups was highly significant. The highest activity of antioxidant enzymes in two cultivar reported at 8mM sodium azide and 10% poly ethylene glycol (Figure 4).

Figure 5 represent a red-blue correlation heatmap is a graphical representation used to visualize the correlation between pairs of variables in a dataset. It's often used in data analysis to quickly identify patterns of positive and negative correlations among variables. The color scale typically ranges from red through white to blue. Furthermore, the intensity of the color in a cell indicates the strength of the correlation. Blue-red heatmap present the interaction and correlation between all studies variables in which fresh weight, shoot length, number of leaves, Chl a, Chl b and carotenoids show a significant negative correlation with PEG. However, antioxidants e.g. POD, CAT, SOD and proline showed a significant positive correlation with PEG. Moreover, all variables show a significant direct correlation with sodium azide.

In addition, the overall effect and interaction between study were evaluated using multivariate analysis of variance (MANOVA) presented in Table 1.

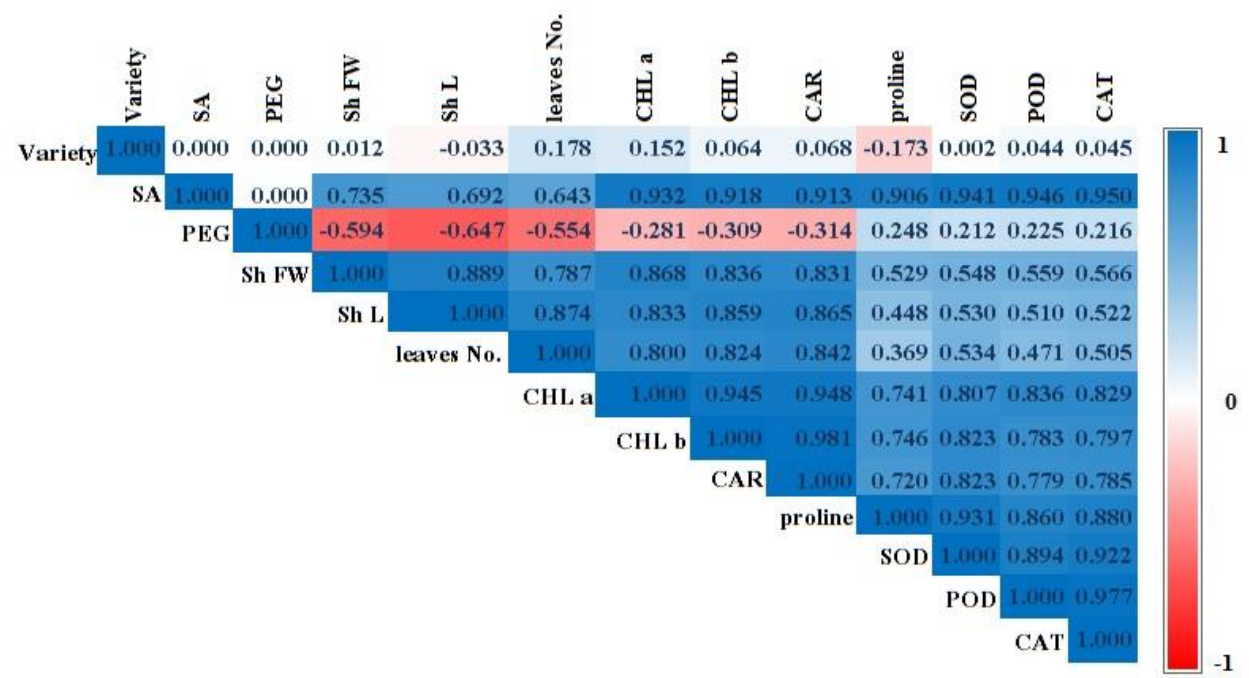

Figure 5. Pearson correlation heatmap showing correlation coefficients-blue and red indicate positive and negative correlation, respectively.

Table 1. Multivariate analysis of variance (MANOVA) presenting the effect of different treatment groups including; sodium azide, poly ethylene glycol, cultivars and their interactions.

Dependent Variable		Source							
		Corrected Model	PEG	Sodium azide	Cultivars	SA*PEG	Cultivars * PEG	Cultivars * SA	Cultivars * SA*PEG
Fresh weight	F	73.9	234.7	349.4	0.2	4.4	5.9	8.7	10.4
	Sig.	<.000***	<.000***	<.000***	0.660^{ns}	0.005**	0.006**	<.001***	<.000***
Number of leaves	F	42.9	122.6	166.2	24.2	18.2	1.4	21.4	2.6
	Sig.	<.000***	<.000***	<.000***	<.000***	<.000***	0.260^{ns}	<.000***	0.052^{ns}
Shoot length	F	201.7	736.3	831.5	3.7	38.2	16.7	21.0	15.5
	Sig.	<.000***	<.000***	<.000***	0.062^{ns}	<.000***	<.000***	<.000***	<.000***
Chl a	F	730.9	491.9	5420.0	287.9	17.8	3.6	90.5	13.9
	Sig.	<.000***	<.000***	<.000***	<.000***	<.000***	0.038*	<.000***	<.000***
Chl b	F	149.4	123.2	1104.1	10.5	15.9	1.3	2.6	1.0
	Sig.	<.000***	<.000***	<.000***	0.003**	<.000***	0.277^{ns}	0.086 ^{ns}	0.399^{ns}
CAR	F	274.2	234.5	1992.1	21.9	30.9	1.9	18.5	5.6
	Sig.	<.000***	<.000***	<.000***	<.000***	<.000***	0.155^{ns}	<.000***	<.001***
Proline	F	420.1	222.9	3067.9	214.0	55.1	4.9	30.6	13.7
	Sig.	<.000***	<.000***	<.000***	<.000***	<.000***	0.013*	<.000***	<.000***
SOD	F	101.6	39.7	815.2	0.0	3.6	0.3	0.5	0.4
	Sig.	<.000***	<.000***	<.000***	0.937^{ns}	0.014*	0.716^{ns}	0.627 ^{ns}	0.6.7833^{ns}
POD	F	483.2	209.5	3866.5	16.0	1.5	2.6	4.4	
	Sig.	<.000***	<.000***	<.000***	<.000***	0.223^{ns}	0.089^{ns}	0.020*	<.000***
CAT	F	128.3	55.7	1022.3	4.6	1.6	1.8	4.3	0.4
	Sig.	<.000***	<.000***	<.000***	0.039*	0.209^{ns}	0.182^{ns}	0.021*	0.812^{ns}

ns, *, ** and *** mean significant at 0.05 , 0.01 and 0.001 level of probability, respectively.

4. Discussion

Results showed that the use of sodium azide had made it possible to improve the tolerance traits through the application of selective pressure in culture conditions. Sodium azide played an important role in improving different characters in sugar beet cultivars. PEG was acting as a nonpenetrating osmotic agent that lowers the water potential of the medium. The two concentrations of PEG, 5% and 10% caused a decrease in shoot fresh weight, length of shoot and number of life in two sugar beet cultivars as well as photosynthetic pigments. Previously, a comparable decrease in plant growth attributes and photosynthetic pigments has been evidenced in sugar beet (Islam *et al.*, 2022; Hussein *et al.*, 2019) under drought stress conditions. Sugar beet is a well-known on of sensitive crops to water stress (Chołuj *et al.*, 2014; Shaw *et al.*, 2002). Also, Blum (2011) reported that the decreasing in leaf area and number of leaves was caused by water stress and by that, the efficiency in light usage becomes decreased. The inhibition of growth could be attributed to diminished photosynthetic activity, osmotic imbalances leading to cell dehydration, heightened cellular toxicity and inadequate nutrient absorption (Sohag *et al.*, 2020; Forni *et al.*, 2017). Drought conditions also impact cell turgor and water absorption, leading to reduced accumulation of cell water, photo-assimilates and metabolites associated with cell elongation (Abd Elbar et al., 2019).

Drought conditions increase the synthesis (ROS), triggering lipid peroxidation, which in turn leads to chlorophyll degradation (Ober *et al.*, 2004; Smirno, 1993). It is widely recognized that drought stress significantly reduces photosynthetic pigments, leading to impaired plant growth and decreased yield (Ashraf & Harris, 2013). A decreased photosynthetic rate is a prevalent issue during drought conditions, primarily attributed to the diminished synthesis of green pigments (Mibei *et al.*, 2017). In this study, there was a significant decrease observed in the contents of chlorophyll a, b, and carotenoids in two cultivars under drought conditions. Research

has indicated that drought stress can affect the photochemical activity of photosystem II (PS II) and the electron requirement for photosynthesis, potentially leading to over-excitation and photo-inhibition damage to the reaction centers of PS II (Akhkha, 2016; Souza *et al.,* 2004).

Results showed that all sodium azide treatments enhanced stress tolerance to drought in studied sugar beet cultivars. These findings are consistent with those reported by Sekhi *et al.* (2022), who observed that sodium azide enhanced the vegetative and vital growth characteristics in strawberry plants experiencing drought stress. In addition to, Vwioko *et al.* (2019) stated that NaN3 promote plant height and number of leaves in okra plants under stress. Also, Kochanová *et al.* (2012) and Al-Qurainy (2009) demonstrated that sodium azide could stimulate the growth and height of *Eruca sativa* and *Diospyros lotus*, respectively. Moreover, the application of sodium azide treatments can help elucidate the impact of drought stress on photosynthetic pigments in maize (Hamideldin & Eliwa, 2015). This could be attributed to the influence of the mutagen on certain molecular characteristics, as indicated by ISSR analysis (Rayan *et al.,* 2014).

In present experiment, sodium azide and drought stress produced a significant increase in proline content compared to the control in both sugar beet cultivars. Drought stress disrupts ROS homeostasis by triggering excessive production, leading to oxidative damage in plants. To mitigate such damage, plants accumulate proline under stressful conditions as a protective measure (Forlani *et al.,* 2019). Addition to that, it has been find that proline can detoxify ROS, particularly hydroxyl radicals, improve photochemical activity in thylakoid membranes, and decrease malondialdehyde formation under various abiotic stresses (Szabados & Savoure, 2010). An increased accumulation of proline concentration serves as an indicator of the plants' adaptive response to drought (da Silva Folli-Pereira *et al.,* 2016).

From our results, all sodium azide treatments trigger the proline content in sugar beet under drought stress. The accumulation of proline serves as a valuable indicator of stress in sugar beet, acting as a signaling molecule to modulate mitochondrial functions, influence cell proliferation, and regulate the expression of specific stress-tolerant genes (Putnik-Deli´c *et al.,* 2010; Szabados & Savoure 2010).

Drought stress raised the activity of antioxidant enzymes (POD, CAT and SOD), whereas the sodium azide treatments highly significant increased the enzymatic antioxidant activity under normal and drought stress. The severity of oxidative damage caused by excessive ROS generation can be regulated by the up-regulation of enzymatic and non-enzymatic antioxidants, such as SOD, CAT, POD, carotenoids, proline, etc. (Islam *et al.,* 2020). There was a strong positive correlation observed between antioxidant enzyme defense activities and increased abiotic stress levels (Ashraf, 2009). During drought stress, plants exhibiting higher antioxidant enzyme activities are regarded as having a superior ability to scavenge free radicals (Wang *et al.,* 2009). In this context, cellular SOD acts as a first-line scavenger of ROS by catalyzing the superoxide radical ($\cdot O_2$) into oxygen and H_2O_2 (Jaleel *et al.,* 2009; Alscher *et al.,* 2002). CAT and POD are detoxifing the H_2O_2 (produced through the dismutation of $\cdot O_2$ in peroxisomes and chloroplasts) to H_2O and O_2 (Bi, 2016). High oxidative stress induced by drought conditions leads to the generation of H_2O_2, which in turn stimulates the production of high antioxidant enzyme activity. Consequently, this enzyme activity may mitigate the adverse effects of abiotic stresses by either serving as a bio-signal or modulating the expression of resistant genes (Alam *et al.* 2014). The activities and participation of various enzymes antioxidant in ROS scavenging can vary depending on factors such as plant species, the severity of stress, and the duration of stress (DaCosta & Huang, 2007).

Sodium azide (NaN_3) is considered the least hazardous and the most effective mutagen, and it has been reported to exhibit mutagenic effects in numerous crop species (Mostafa, 2011). Sekhi *et al.* (2022) showed that sodium azide could be increasing the antioxidants' resistance to drought stress in strawberry. Also, Vwioko *et al.* (2019) reported that the sodium azide priming also enhanced the activities and gene expression level of antioxidant enzymes (ascorbate peroxidase, APX; catalase, CAT) under stress. The introduction of sodium azide to the callus of the black seed plant resulted in an elevation of antioxidant levels (Iqbal *et al.*, 2019). In addition to, Elfeky *et al.* (2014) it has been reported that increasing the concentrations and duration of soaking of sodium azide stimulated the antioxidant defense system of *Helianthus annuus*.

5. Conclusion

Presoaking of sugar beet seeds in sodium azide play a vital role to enhance drought stress. Different concentrations of poly ethylene glycol (5% or 10%) caused a decreasing in water potential of the medium. It leads to shortage in shoot length, fresh weight, number of leaves and photosynthetic pigments. While, it produced limited raising in proline content and enzymatic antioxidant (POD, SOD and CAT). The application by sodium azide raised the growth parameter in sugar beet cultivars at unstressed and stressed conditions. In addition to, it triggered the proline content and enzymatic antioxidant and the maximum values showed at 8mM sodium azide and 10% PEG.

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