



Effects of Exogenous Auxins on the in Vitro Growth and Physiological Responses of Two Unnamed Tomato (*Solanum lycopersicum* L.) Genotypes Under PEG-Induced Osmotic Stress

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<https://doi.org/10.18280/ijdne.210910>

ABSTRACT

Received: 17 June 2026

Revised: 18 August 2026

Accepted: 25 August 2026

Available online: 30 September 2026

Keywords:

auxins, chlorophyll, drought stress, indole-3-butyric acid, polyethylene glycol-6000, proline, Solanum lycopersicum, tomato tissue culture

This study investigated the effects of polyethylene glycol (PEG)-induced drought stress and exogenous auxin treatments on the in vitro growth, chlorophyll content, and proline accumulation of two unnamed Iraqi tomato (*Solanum lycopersicum* L.) genotypes. Nodal explants were cultured on Murashige and Skoog (MS) medium under nine treatment combinations: 0% PEG without auxin, 5% PEG without auxin, 7% PEG without auxin, and 5% or 7% PEG supplemented with 1.0 mg L⁻¹ indole-3-acetic acid (IAA), indole-3-butyric acid (IBA), or naphthalene acetic acid (NAA). PEG stress reduced growth in a trait-dependent manner, with the strongest inhibition under 7% PEG. Root number and root length were highest under 5% PEG + IBA, indicating that IBA improved root initiation and elongation under moderate osmotic stress. Shoot length was highest in the unstressed control, confirming the sensitivity of shoot elongation to PEG-induced water deficit. With an increase in PEG concentration, the chlorophyll content decreased gradually and in a linear fashion, with IAA and IBA having higher Soil Plant Analysis Development (SPAD) values than NAA, but not reaching the control. The accumulation of proline was highly elevated under stress and was highest in the plantlets treated with 7% PEG + IBA, indicating better osmotic adjustment. Under the tested PEG conditions, IBA-containing media were associated with higher root number and root length, particularly at 5% PEG. The findings revealed that genotype B exhibited higher values for selected growth and physiological variables than genotype A under the tested conditions.

1. INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is one of the most economically important vegetable crops worldwide due to its nutritional value, antioxidant properties, and diverse applications in both fresh and processed forms. Its cultivation is significant for the local markets in Iraq. Abiotic stress, particularly water deficit under arid and semi-arid conditions, is increasingly limiting tomato crop performance in the country [1, 2]. The effects of drought stress include interference with water relations, nutrient uptake, membrane stability, and photosynthetic activity, which leads to reduced plant growth and productivity [3]. At the cellular level, water deficit is associated with stomatal limitation, reduced carbon assimilation, and accumulation of excessive reactive oxygen species (ROS), which can cause harmful effects on lipids, proteins, and nucleic acids when antioxidant defenses are impaired [4].

Tomato is normally drought-sensitive, and drought tolerance is genotype-dependent. In recent years, tomato landraces were tested, and the morphological and physiological traits were found to be able to differentiate

tolerant from sensitive genotypes when subjected to limited irrigation [5]. The in vitro tissue culture condition is a controlled environment for testing the initial responses of plants to water stress, minimizing environmental variation and permitting the precise control of the stress intensity. Polyethylene glycol (PEG-6000) has been widely employed as an osmotic stress agent because it is an effective way of lowering the water potential of the culture medium, but it does not easily enter into plant tissues [6, 7]. These are advantageous in propagating plants, stress physiology, and early identification of stress-adapted lines [8].

Root development is key to drought adaptation because it is a factor that affects plant uptake of water and nutrients during drought. Auxins are one of the important plant growth regulators involved in initiation of roots, formation of lateral roots, cell expansion, and morphogenesis. Indole-3-acetic acid (IAA), indole-3-butyric acid (IBA), and naphthalene acetic acid (NAA) are some of the phytohormones that have been used in tomato and other species to enhance rooting and establishment of plantlets [9, 10]. Furthermore, auxin metabolism and signaling have been found to be associated with developmental plasticity under stress conditions, while

recent molecular studies have revealed that auxin-like pathways are involved in the regulation of growth in response to stress [11, 12].

Biochemical markers, in addition to morphological characters, are valuable indicators of adaptation to stress. The accumulation of proline in plants has been reported under drought conditions, and this accumulation is associated with osmotic adjustment, membrane protection, redox balance, and scavenging of ROS [13]. In tomato, the proline-related responses have been related to stress tolerance differences between genotypes [14, 15]. Drought stress on tomato has been a focus of many studies, but simple PEG-induced in vitro drought stress, auxin application, chlorophyll retention, and proline accumulation studies have not been carried out together.

Thus, the present study was conducted to assess the effect of drought stress imposed by PEG and three auxins on the in vitro growth, chlorophyll content, and proline accumulation.

2. MATERIALS AND METHODS

2.1 Plant materials and explant preparation

Seeds of two unnamed tomato genotypes locally grown in Iraq (*Solanum lycopersicum* L.) were acquired from the Iraqi Ministry of Agriculture. However, formal cultivar registration/accession information was not available to the investigators. Therefore, the materials are called genotype A and genotype B throughout the manuscript. Therefore, the results of this study are offered as preliminary screening of two unnamed tomato genotypes/accessions, and conclusions regarding the genetic background or drought tolerance of the tomato cultivars are not drawn. The seeds were washed under running tap water, surface sterilized in 70% (v/v) ethanol for 1 min, and then surface-sterilized in 2% (v/v) sodium hypochlorite for 10 min. The seeds were sterilized with sterile distilled water thrice and germinated aseptically on Murashige and Skoog (MS) medium without hormones [16]. After two weeks, healthy nodal segments of approximately 1.0–1.5 cm were excised and used as explants for the experimental treatments [17].

2.2 Culture medium and growth conditions

All explants were cultured on MS basal medium supplemented with 30 g L⁻¹ sucrose and solidified with 7 g L⁻¹ agar. About 30 mL of MS medium was added into 250 mL culture jars, and one explant was placed in each jar. The pH of the medium was adjusted to 5.8 before autoclaving at 121 °C for 20 min. Cultures were incubated at 25 ± 2 °C under a 16 h photoperiod with a light intensity of approximately 2000 lux [8].

2.3 Polyethylene glycol and auxin treatments

This is an exploratory comparison of nine treatment combinations. PEG-6000 was used to induce osmotic stress at 0%, 5%, and 7% (w/v). The osmotic potentials were estimated to be approximately -0.027 MPa for 5% PEG-6000 and -0.05 MPa for 7% PEG-6000 at 25 °C using the formula given by Michel and Kaufmann [6]. Within the parameters of this in vitro system, the 5% and 7% PEG treatments were considered moderate and severe osmotic stress, respectively. The

experimental treatment combinations were: control (0% PEG without auxin), 5% PEG without auxin, 7% PEG without auxin, 5% PEG + IAA, 5% PEG + IBA, 5% PEG + NAA, 7% PEG + IAA, 7% PEG + IBA, and 7% PEG + NAA. Auxins were added at a single concentration of 1.0 mg L⁻¹ after filter sterilization. The inclusion of PEG-only treatments allowed separation of the direct osmotic-stress effect from the auxin-mediated response. Similar use of plant growth regulators in micropropagation systems has been reported for several horticultural species [10, 18]. The approximate PEG-induced osmotic potentials were calculated using the method of Michel and Kaufmann [6] as the approximate PEG induced osmotic potentials were calculated as -0.05 MPa and -0.08 MPa at 25 °C for 5% and 7% PEG, respectively, following the approach of Michel and Kaufmann [6]. In this study, 5% PEG and 7% PEG were considered as moderate and severe stress, respectively, in the in vitro system. for 7% PEG at 25 °C. The 5% and 7% PEG used in this study were defined as moderate and severe stress, respectively, and these are defined relative to this in vitro system.

2.4 Experimental design

The experiment was replicated twice in a completely randomized design (CRD) with two genotypes and nine treatment combinations. Each genotype-treatment combination had five replications in each run, with one explant per culture jar. Experimental run was included in the statistical model to account for between-run variation. The interaction between experimental run and treatment was examined before determining whether data from the two runs could be combined.

Each run × treatment combination had a treatment × run interaction that was not significant ($p > 0.05$), and hence data were pooled across the two runs, resulting in $n = 10$ for each genotype × treatment combination in the pooled analysis.

2.5 Growth and physiological measurements

The number of roots per plantlet, root length (cm), and shoot length (cm) were measured after 4 weeks of culture. The lengths of roots and shoots were determined with a digital caliper. Fully expanded leaves were used to estimate the chlorophyll content with a Soil Plant Analysis Development (SPAD) relative chlorophyll meter, and chlorophyll content was reported in SPAD units. The proline content was measured by the ninhydrin method of Bates et al. [19]. Fresh leaf tissue (0.5 g) was homogenized in 3% sulfosalicylic acid and centrifuged at 10,000 rpm for 10 min. Two mL of the supernatant was combined with 2 mL acid ninhydrin and 2 mL glacial acetic acid and heated to 100 °C for 1 h, cooled on ice, and extracted with 4 mL of toluene. The absorbance at 520 nm was measured, and proline concentration was determined from a standard curve and reported as $\mu\text{mol g}^{-1}$ fresh weight.

2.6 Statistical analysis

Data are presented as mean ± standard error (SE). Two-way analysis of variance (ANOVA) was used to analyze the Genotype, treatment combination, and the interaction between these. In all cases of pairwise comparisons, $p < 0.05$, means were compared using Tukey's HSD test. Model assumptions were assessed using Shapiro–Wilk tests and Q–Q plots of residuals for normality and Levene's test for homogeneity of

variance. Root number was additionally evaluated as a count response using a generalized linear model with an appropriate distribution based on dispersion diagnostics. Data were presented in graphs and analyzed statistically using the Statistical Package for Social Sciences (SPSS) version 26 and plotting software, such as GraphPad Prism.

3. RESULTS

The results of two-way ANOVA (Table A1) revealed highly significant effects for genotype, as well as for treatment, across all measured traits. For root number, significant effects were detected for genotype ($F_{1,162} = 13.63$, $p < 0.001$) and treatment ($F_{8,162} = 35.66$, $p < 0.001$), whereas the genotype $\times$ treatment interaction was not significant ($F_{8,162} = 0.07$, $p > 0.05$). Significant genotype and treatment effects were also observed for root length ($F_{1,162} = 7.76$, $p = 0.006$; $F_{8,162} = 33.87$, $p < 0.001$), shoot length ($F_{1,162} = 7.98$, $p = 0.005$; $F_{8,162} = 54.64$, $p < 0.001$), chlorophyll content ($F_{1,162} = 29.93$, $p < 0.001$; $F_{8,162} = 94.61$, $p < 0.001$), and proline content ($F_{1,162} = 11.34$, $p < 0.001$; $F_{8,162} = 29.09$, $p < 0.001$). There was no significant interaction between genotype and treatment for root length, shoot length, chlorophyll content, or proline content ($p > 0.05$).

3.1 Root number

Root number was distinctly different by genotype and treatment, as shown in Table 1 and Figure 1. Genotype B had a higher number of roots than genotype A in all the treatments. While root production was not affected by moderate PEG stress, severe stress at 7% PEG decreased root production compared to the unstressed control. The highest root numbers were recorded under 5% PEG + IBA in genotype A (6.8 ± 0.5) and genotype B (7.4 ± 0.4). The lowest root numbers were recorded when the plants were exposed to 7% PEG + NAA, which showed the weakness of NAA compared to IBA under severe osmotic stress.

Table 1. Effect of polyethylene glycol (PEG) and auxin treatments (1.0 mg L^{-1}) on root number

Treatment	Genotype A	Genotype B
Control	$4.2 \pm 0.3 \text{ c}$	$4.8 \pm 0.2 \text{ c}$
5% PEG	$4.5 \pm 0.3 \text{ bc}$	$5.0 \pm 0.3 \text{ bc}$
7% PEG	$3.0 \pm 0.2 \text{ d}$	$3.5 \pm 0.2 \text{ d}$
5% PEG + IAA	$5.1 \pm 0.4 \text{ b}$	$5.6 \pm 0.3 \text{ b}$
5% PEG + IBA	$6.8 \pm 0.5 \text{ a}$	$7.4 \pm 0.4 \text{ a}$
5% PEG + NAA	$4.9 \pm 0.3 \text{ bc}$	$5.2 \pm 0.3 \text{ bc}$
7% PEG + IAA	$3.2 \pm 0.3 \text{ d}$	$3.8 \pm 0.2 \text{ d}$
7% PEG + IBA	$5.4 \pm 0.4 \text{ b}$	$6.1 \pm 0.3 \text{ b}$
7% PEG + NAA	$2.9 \pm 0.2 \text{ d}$	$3.4 \pm 0.2 \text{ d}$

Note: Values are presented as mean $\pm$ SE ($n = 10$). Different letters within the same column indicate significant differences at $p \leq 0.05$. Letters were generated by Tukey's HSD test. IAA: Indole-3-acetic acid; IBA: Indole-3-butyric acid; NAA: Naphthalene acetic acid.

3.2 Root length

High PEG stress had a significant effect on root length (Table 2 and Figure 2). The longest roots were observed in the plantlets grown with PEG 5% + IBA, which measured $6.2 \pm 0.5 \text{ cm}$ in genotype A and $6.7 \pm 0.4 \text{ cm}$ in genotype B. The lowest root length was found under the 7% PEG + NAA condition, on the other hand. The results indicate that root length was strongly reduced under 7% PEG, whereas 5% PEG

alone produced values comparable to or slightly higher than the unstressed control. Meanwhile, severe PEG stress significantly reduced root elongation compared to IBA, IAA, and NAA.

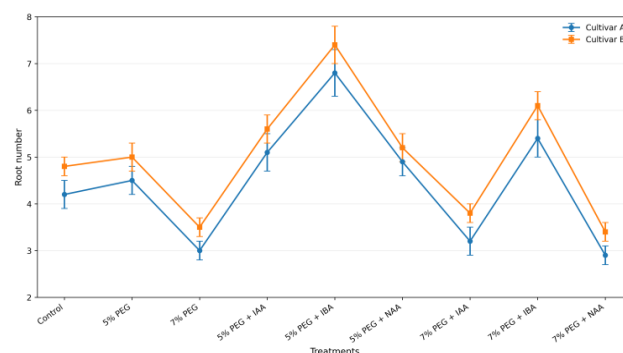


Figure 1. Effect of polyethylene glycol (PEG)-induced drought stress and auxin treatments on root number

Table 2. Effect of polyethylene glycol (PEG) and auxin treatments (1.0 mg L^{-1}) on root length (cm)

Treatment	Genotype A	Genotype B
Control	$5.8 \pm 0.4 \text{ bc}$	$6.1 \pm 0.3 \text{ bc}$
5% PEG	$5.9 \pm 0.4 \text{ b}$	$6.3 \pm 0.3 \text{ b}$
7% PEG	$3.5 \pm 0.3 \text{ d}$	$4.0 \pm 0.3 \text{ d}$
5% PEG + IAA	$6.0 \pm 0.4 \text{ ab}$	$6.4 \pm 0.3 \text{ ab}$
5% PEG + IBA	$6.2 \pm 0.5 \text{ a}$	$6.7 \pm 0.4 \text{ a}$
5% PEG + NAA	$5.7 \pm 0.3 \text{ bc}$	$6.0 \pm 0.3 \text{ bc}$
7% PEG + IAA	$4.1 \pm 0.3 \text{ cd}$	$4.6 \pm 0.3 \text{ cd}$
7% PEG + IBA	$4.1 \pm 0.3 \text{ cd}$	$4.6 \pm 0.3 \text{ cd}$
7% PEG + NAA	$2.3 \pm 0.2 \text{ e}$	$2.8 \pm 0.2 \text{ e}$

Note: Values are presented as mean $\pm$ SE ($n = 10$). Different letters within the same column indicate significant differences at $p \leq 0.05$. Letters were generated by Tukey's HSD test. IAA: Indole-3-acetic acid; IBA: Indole-3-butyric acid; NAA: Naphthalene acetic acid.

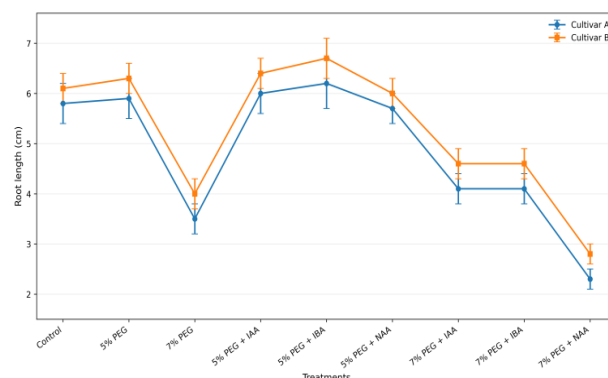


Figure 2. Effect of polyethylene glycol (PEG)-induced drought stress and auxin treatments on root length of two tomato genotypes

3.3 Shoot length

As presented in Table 3 and Figure 3, shoot length differed among the PEG treatments. The longest shoots were recorded in the unstressed control, with mean values of $7.1 \pm 0.5 \text{ cm}$ and $7.6 \pm 0.4 \text{ cm}$ for genotypes A and B, respectively. Shoot length decreased markedly under 7% PEG, reaching $3.3 \pm 0.3 \text{ cm}$ in genotype A and $3.7 \pm 0.2 \text{ cm}$ in genotype B. The auxin treatments did not restore shoot length to control levels, indicating that exogenous auxin application did not enhance shoot elongation under the conditions of this experiment.

Table 3. Effect of polyethylene glycol (PEG) and auxin treatments (1.0 mg L⁻¹) on shoot length (cm)

Treatment	Genotype A	Genotype B
Control	7.1 ± 0.5 a	7.6 ± 0.4 a
5% PEG	6.5 ± 0.4 b	6.9 ± 0.3 b
7% PEG	3.3 ± 0.3 d	3.7 ± 0.2 d
5% PEG + IAA	6.3 ± 0.4 b	6.8 ± 0.3 b
5% PEG + IBA	6.3 ± 0.4 b	6.8 ± 0.3 b
5% PEG + NAA	6.0 ± 0.3 b	6.3 ± 0.3 b
7% PEG + IAA	3.5 ± 0.3 cd	3.9 ± 0.2 cd
7% PEG + IBA	3.5 ± 0.3 cd	3.9 ± 0.2 cd
7% PEG + NAA	3.2 ± 0.2 d	3.6 ± 0.2 d

Note: Values are presented as mean ± SE (n = 10). Different letters within the same column indicate significant differences at $p \leq 0.05$. Letters were generated by Tukey's HSD test. IAA: Indole-3-acetic acid; IBA: Indole-3-butyric acid; NAA: Naphthalene acetic acid.

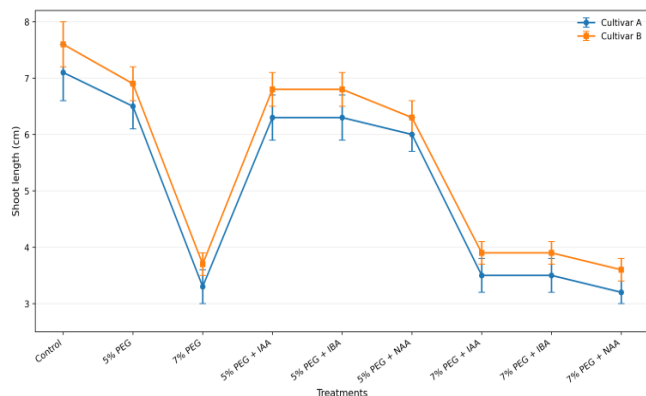


Figure 3. Effect of polyethylene glycol (PEG)-induced drought stress and auxin treatments on shoot length

3.4 Chlorophyll content

The percentage of chlorophyll, based on SPAD index measurements, decreased as the PEG concentration increased (Table 4 and Figure 4). The highest SPAD value was found in the control plantlets, and the lowest was observed in 7% PEG + NAA. With less than 5% PEG, IAA and IBA kept their chlorophyll content higher than NAA, but not higher than the unstressed control. The higher SPAD values for genotype B as compared to genotype A in all treatments suggest that genotype B retains more chlorophyll than genotype A under these in vitro conditions, but does not necessarily have better drought tolerance.

Table 4. Effect of polyethylene glycol (PEG) and auxin treatments (1.0 mg L⁻¹) on chlorophyll content

Treatment	Genotype A	Genotype B
Control	38.5 ± 1.2 a	41.3 ± 1.1 a
5% PEG	35.0 ± 1.0 b	37.2 ± 0.9 b
7% PEG	25.0 ± 0.8 d	27.0 ± 0.7 d
5% PEG + IAA	33.2 ± 1.0 bc	35.7 ± 0.9 bc
5% PEG + IBA	33.2 ± 1.0 bc	35.7 ± 0.9 bc
5% PEG + NAA	32.0 ± 0.9 c	34.0 ± 0.8 c
7% PEG + IAA	24.6 ± 0.8 d	26.9 ± 0.7 d
7% PEG + IBA	24.6 ± 0.8 d	26.9 ± 0.7 d
7% PEG + NAA	21.4 ± 0.7 e	23.1 ± 0.6 e

Note: Values are presented as mean ± SE (n = 10). Different letters within the same column indicate significant differences at $p \leq 0.05$. Letters were generated by Tukey's HSD test. IAA: Indole-3-acetic acid; IBA: Indole-3-butyric acid; NAA: Naphthalene acetic acid.

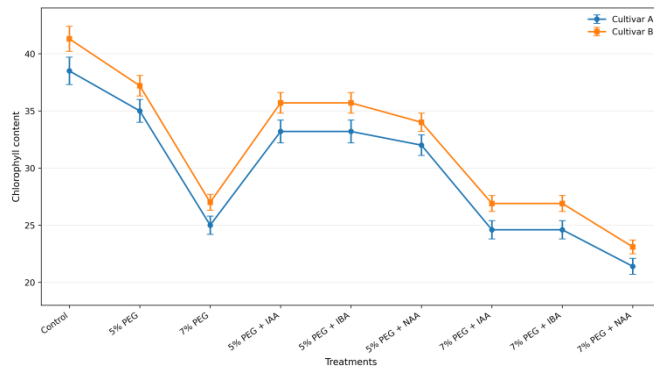


Figure 4. Effect of polyethylene glycol (PEG)-induced drought stress and auxin treatments on chlorophyll content

3.5 Proline content

The higher the PEG concentration, the higher the amount of proline, as depicted in Table 5. The lowest proline content was obtained in the unstressed control, and stressed plantlets had significantly higher proline levels. The highest level of proline was found at 7% PEG + IBA in genotypes A ($4.2 \pm 0.3 \mu\text{mol g}^{-1} \text{FW}$) and B ($4.5 \pm 0.3 \mu\text{mol g}^{-1} \text{FW}$). The results obtained suggest that in the case of severe stress, IBA can improve osmotic adjustment. A few IAA and IBA values are equal to one decimal place shown in the tables, and these should be taken as being equal at the displayed one decimal point. The effects of PEG-6000 concentration and plant growth regulators on proline content are presented in Figure 5.

Table 5. Effect of polyethylene glycol (PEG) and auxin treatments (1.0 mg L⁻¹) on proline content ($\mu\text{mol g}^{-1} \text{FW}$)

Treatment	Genotype A	Genotype B
Control	1.3 ± 0.1 d	1.5 ± 0.1 d
5% PEG	2.8 ± 0.2 c	3.1 ± 0.2 c
7% PEG	3.8 ± 0.3 b	4.2 ± 0.3 b
5% PEG + IAA	3.1 ± 0.2 bc	3.6 ± 0.2 bc
5% PEG + IBA	3.1 ± 0.2 bc	3.6 ± 0.2 bc
5% PEG + NAA	2.9 ± 0.2 bc	3.3 ± 0.2 bc
7% PEG + IAA	4.0 ± 0.3 ab	4.3 ± 0.3 ab
7% PEG + IBA	4.2 ± 0.3 a	4.5 ± 0.3 a
7% PEG + NAA	3.5 ± 0.2 b	3.9 ± 0.2 b

Note: Values are presented as mean ± SE (n = 10). Different letters within the same column indicate significant differences at $p \leq 0.05$. Letters were generated by Tukey's HSD test. IAA: Indole-3-acetic acid; IBA: Indole-3-butyric acid; NAA: Naphthalene acetic acid.

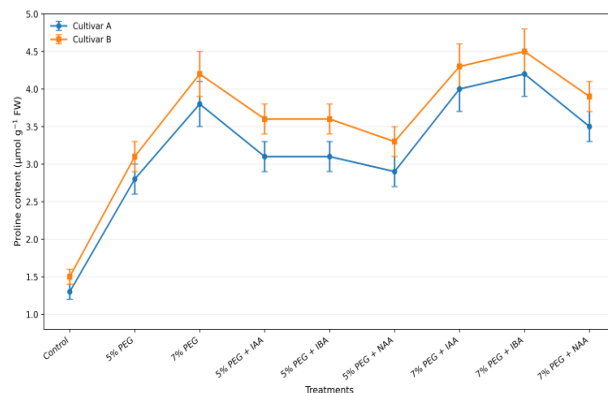


Figure 5. Effect of polyethylene glycol (PEG)-induced drought stress and auxin treatments on proline accumulation

The Shapiro–Wilk test for normality and Levene's test for homogeneity of variances were used to assess the statistical analysis. The results showed that the root length ($W = 0.991$, $p = 0.281$), shoot length ($W = 0.988$, $p = 0.143$), chlorophyll content ($W = 0.992$, $p = 0.388$), and proline content ($W = 0.989$, $p = 0.181$) were normally distributed. But for root number, the deviation from normality was great ($W = 0.983$, $p = 0.029$). Levene's test revealed homogeneous variances among treatments for root number ($F = 1.63$, $p = 0.062$), root length ($F = 1.49$, $p = 0.105$), and chlorophyll content ($F = 1.26$, $p = 0.225$). However, significant heterogeneity of variances was found for shoot length ($F = 1.84$, $p = 0.027$) and proline content ($F = 1.89$, $p = 0.022$).

4. DISCUSSION

The current study provides evidence that the osmotic stress caused by PEG had a specific effect on tomato plantlets. Root number, root length, shoot length, and chlorophyll content were significantly decreased by severe stress at 7% PEG, indicating that water deficit negatively affects morphogenesis and photosynthetic potential *in vitro*. Generally, the responses are in line with plant physiology, which includes a decrease in water potential, a decrease in CO_2 assimilation, and an increase in oxidative stress on plant cells under drought conditions [3, 4]. PEG-based *in vitro* screening is therefore useful as it provides a controlled reduction of water availability to plantlets, as the soil is not a source of variability [6, 7]. Some root traits showed a mild stimulatory response under 5% PEG; in particular, under the provision of IBA. This reaction may be considered to be low-intensity stress adaptation, not necessarily better performance in all growth parameters. In plants, environmental hormesis is defined as an effect in which low-intensity levels of stress induce protective or growth-related responses but higher intensity levels cause inhibitory ones [20]. In this situation, the hormetic effect was most pronounced in root number and length, and not in shoot length and chlorophyll content; this highlights the need to consider each trait individually.

IBA (1.0 mg L^{-1}) gave the maximum rooting response in comparison to the other two auxins under the 5% PEG condition. This agrees with the auxin function in the initiation of adventitious and lateral roots, meristem activity, and root elongation [9, 10]. Due to moderate osmotic pressure caused by PEG + IBA, root number and root length were increased over the unstressed control under PEG + IBA, thus indicating that IBA has a role in directing plantlet growth towards root development. The root improvement is agronomically important since a better root system is one of the primary adaptive traits determining water deficit tolerance [5]. Recent research on auxin metabolism also reinforces the idea that auxin signaling plays a role in developmental plasticity in response to stress [12]. Root traits were not the same as shoot length. The control had the highest shoot length, with the lowest values under severe PEG treatment. Though IBA enhanced rooting, there was no enhancement in shoot length above control, suggesting shoot growth remained sensitive to PEG-induced osmotic limitation. This difference in root/shoot responses is significant since drought-assisted plantlets could invest more resources into the root than the shoot under stress. Such reorganization of growth allocation has been reported for plants facing drought stress where water-saving mechanisms (smaller shoots) are coupled with maintenance or stimulation

of root-related attributes [21].

The greater rooting response seen with IBA compared to IAA or NAA might be due to differences in auxin stability, auxin transport, and metabolism, and not necessarily due to the general superiority of IBA. IAA is metabolized or conjugated at a relatively high rate, while IBA may be more stable and serve as a precursor for the formation of IAA, thus maintaining auxin activity in the root-forming tissues [11, 12]. Such differences can change the nature of the auxin signal and/or receptor-mediated responses. In addition, the higher level of proline when PEG + IBA (auxin–ABA crosstalk in osmotic stress signaling) may also be due to this reason, but a measurement of ABA was not performed here; thus, it is still a hypothesis [12, 21]. The effects on root traits were more pronounced than on shoot length or chlorophyll, which were still limited by PEG-induced water deficit, indicating direct sensitivity of these processes to auxin. Since no unstressed control (auxin only) was used, stress-specific auxin responses were not distinguished from the auxin effects under non-stress conditions in this experiment. The decrease in chlorophyll content with increasing PEG concentration suggests a negative effect of PEG on photosynthetic status under stress. This reduction is consistent with reports that drought and PEG-induced osmotic stress reduce chlorophyll metabolism and photosynthetic performance [22, 23]. IAA- and IBA-treated plantlets generally showed higher SPAD values than NAA-treated plantlets at the same PEG level, but their values remained below those of the unstressed control. Thus, the protective action of auxins against the loss of chlorophyll can be summarized as "partial" instead of "total". Broad phenotyping efforts also show that chlorophyll-related traits are highly responsive to drought and can have large inter-genotype variation, as shown by Denes et al. [24] and Grozeva et al. [5]. The accumulation of proline was significantly higher under PEG stress and was highest for 7% PEG + IBA. This pattern is consistent with the proposed functions of proline as an osmoprotectant in osmotic adjustment, as a protein stabilizer, as a protectant of membranes, and as a redox buffer [13, 19]. A similar increase in proline has been mentioned for tomato cultivars under drought stress, and the proline content in tolerant cultivars is higher than in sensitive cultivars [14, 15].

In a preliminary screening, genotype B had higher proline values in *in vitro* conditions, but this is not the only evidence of superior drought tolerance.

In general, the IBA effects were more pronounced on root traits and proline accumulation, while effects were less pronounced on shoot length and chlorophyll content. This separation would enhance the biological interpretation of the experiment: IBA did not remove PEG-induced stress, but it supported plantlets' adaptive responses that are vital to plant development, such as root development and osmotic adjustment. These results could help optimize the *in vitro* system for testing tomatoes under water deficit conditions. Additional studies should incorporate molecular markers, antioxidant enzyme assays, and acclimatization or greenhouse testing to determine if the *in vitro* responses observed will result in drought tolerance in the whole plant.

5. CONCLUSION

The study revealed that the *in vitro* tomato plantlets were greatly influenced by PEG-induced osmotic stress. Root and

shoot growth decreased under severe stress (7% PEG), and the chlorophyll content decreased, but proline content increased as the stress intensified. Among the three auxins tested, IBA gave the maximum root number and root length at the tested concentration (1.0 mg L^{-1}) in both 5% and 7% PEG solutions, and the highest proline accumulation in 7% PEG solution. Shoot length and chlorophyll content were the highest in the control condition, suggesting that the positive effect of IBA on these characteristics was not consistent and specific. Genotype B showed higher values for several measured growth and physiological parameters under the tested in vitro conditions; however, these observations alone are insufficient to establish superior drought tolerance. The study's findings suggest that 5% PEG + 1.0 mg L^{-1} IBA is more suitable for root development under moderate in vitro drought stress, whereas 7% PEG + IBA is more suitable for osmotic adjustment by increasing proline level.

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APPENDIX

Table A1. Analysis of variance (ANOVA) results for growth and biochemical parameters of tomato plantlets under polyethylene glycol (PEG)-induced drought stress

Trait	Source	df	SS	MS	F	p-value
Root Number	Treatment	8	267.878	33.485	35.664	<0.001
	Genotype	1	12.800	12.800	13.633	<0.001
	Treatment × Genotype	8	0.500	0.063	0.067	0.9998
	Error	162	152.100	0.939	—	—
	Total	179	433.278	2.420	—	—
Root Length	Treatment	8	295.078	36.885	33.874	<0.001
	Genotype	1	8.450	8.450	7.760	0.006
	Treatment × Genotype	8	0.300	0.038	0.034	1.000
	Error	162	176.400	1.089	—	—
	Total	179	480.228	2.965	—	—
Shoot Length	Treatment	8	439.578	54.947	54.644	<0.001
	Genotype	1	8.022	8.022	7.978	0.005
	Treatment × Genotype	8	0.178	0.022	0.022	1.000
	Error	162	162.900	1.006	—	—
	Total	179	610.678	3.412	—	—
Chlorophyll Content	Treatment	8	5790.200	723.775	94.611	<0.001
	Genotype	1	228.939	228.939	29.927	<0.001
	Treatment × Genotype	8	4.311	0.539	0.070	1.000
	Error	162	1239.300	7.650	—	—
	Total	179	7262.750	40.574	—	—
Proline Content	Treatment	8	124.111	15.514	29.089	<0.001
	Genotype	1	6.050	6.050	11.344	<0.001
	Treatment × Genotype	8	0.400	0.050	0.094	0.999
	Error	162	86.400	0.533	—	—
	Total	179	216.961	1.212	—	—