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MORPHO-BIOCHEMICAL RESPONSE OF BLUEBERRY CULTIVAR HORTBLUE PETITE UNDER DROUGHT STRESS INDUCED BY PEG 6000

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Abstract

The in vitro response of the blueberry cultivar Hortblue Petite to drought stress induced by PEG 6000 was investigated in this study. PEG was added to the culture media before pH adjustment at the following concentrations: 0 g/L, 10 g/L, 20 g/L, 30 g/L, 40 g/L, 50 g/L. The culture medium used was Woody Plant Medium (WPM) with 100 mg/l Sequestren 138, 1 mg/L zeatin (Z), and a pH of 5. After 80 days of in vitro culture, the average number of shoots, shoot length, fresh weight (FW), dry weight (DW), water content (WC), chlorophyll (Chl a, Chl b), carotenoids (Caro), proline (Pro), total soluble sugars (TSS), total phenolic compounds (TPC), total flavonoids (TFC), malondialdehyde (MDA), and hydrogen peroxide (H_2O_2) were evaluated. Drought stress induced by PEG increased the number of proliferated shoots but harmed shoot length. A reduction in the content of photosynthetic pigments was also observed. Osmolytes, oxidative stress markers, and antioxidant compounds also indicated the effect of drought stress on the Hortblue Petite blueberry cultivar.

Key words*: drought, hydrogen peroxide, malondialdehyde, proline, V. corymbosum.*

INTRODUCTION

Highbush blueberry (*Vaccinium corymbosum* L.) is the most commonly cultivated, commercially important, and biologically valuable species of the genus *Vaccinium*. In recent years, blueberries have become increasingly popular among consumers worldwide due to their flavor, high nutritional value, and health benefits (Ruzić et al., 2012; Mazurek et al., 2021; Ciucu-Paraschiv et al., 2023; Hera et al., 2023; Muñoz-Fariña et al., 2023; Shi et al., 2023). At the same time, highbush blueberry is one of the most sensitive species to water shortages in soils due to its superficial root system (Bryla & Strik, 2007; Sandoval et al., 2024). It is recognized that there are many varieties of *V. corymbosum*, but their response to drought depends largely on the genetic characteristics of each variety (Calderón-Orellana et al., 2023).

Drought is an important abiotic stress, caused by water imbalance in the ecosystem, which can have severe consequences in plants and lead to significant losses in the production of woody species (Vuksanović et al., 2022). Water stress affects the normal functionality of plants and induces morphological, physiological, and biochemical changes. A detailed understanding of the patterns and response mechanisms of plants to water stress is crucial for predicting the functionality and resilience of plants in the face of increasingly frequent drought episodes (Sun et al., 2020).

The in vitro screening condition is considered beneficial compared to field screening, although the drought effect may not be replicated at all stages of development through this method. Preliminary reports can predict the plant response to stress, and subsequent validation of responses to stress can be conducted under field conditions (Sahu et al., 2023).

Polyethylene glycol (PEG), a high molecular weight compound, is used to induce drought stress in different plants species under in vitro conditions such as: wild cherry (Vuksanović et al., 2022), fox grapes (Bilir et al., 2022), highbush blueberry (Molnar et al., 2022), cotton (Jaafar et al., 2021), vanilla (Martínez-Santos et al., 2021), sugarcane (Hernández Pérez et al., 2021), stevia (Ahmad et al., 2020), sweet violet (Darvishani et al., 2020), tomato (Naveed et al., 2019), olive (Silvestri et al., 2017).

Several biological parameters change while plants are subjected to drought stress and their study provides important data to understand the mechanisms by which plants can tolerate this phenomenon (Tafreshi et al., 2021). Along with the morphological changes of plants under drought stress, some biochemical parameters can define the resistance of plants to drought stress: photosynthetic pigments, antioxidant compounds, oxidative stress markers, or osmolytes (Coşkun, 2023; Eisa et al., 2023; Habuš Jerčić et al., 2023). In this context, the objective of this study was to evaluate the morphological and biochemical responses in in vitro blueberry cultivar Hortblue Petite shoots under drought stress conditions induced by PEG.

MATERIALS AND METHODS

In vitro culture and drought stress

Blueberry shoots (Hortblue Petite cv.) from the in vitro multiplication phase were used in this study. The shoots were propagated on Woody Plant Medium (WPM) (Lloyd & McCown, 1980) supplemented with 100 mg/L Sequestrene 138 and 1 mg/L zeatin (Z) according to the protocol described by Clapa et al., 2018. The experimental design included using different concentrations of PEG 6000 as follows: 0 g/L, 10 g/L, 20 g/L, 30 g/L, 40 g/L, 50 g/L. PEG 6000 was added to the culture media before adjusting the pH and then autoclaved. The culture medium was solidified with 0.5% (w/v) plant agar. The pH of the media was adjusted to 5 before adding agar.

The culture medium was distributed in glass culture jars (capacity 720 mL; diameter 9 cm and height 13.5 cm) which were closed with transparent polyethylene caps. Then, 15 explants (each 1.5-2 cm long) were inoculated into each culture jar. After inoculation, the culture vessels were incubated in the growth room with a controlled environment (21 ± 1°C, 32.4 $mmol \cdot m^{-2} \cdot s^{-1}$, 16-h photoperiod). All the chemicals and reagents were purchased from Duchefa BiochemieBV, The Netherlands.

Growth parameters

The *in vitro* growth response to drought stress was evaluated after 12 weeks by measuring the following parameters: shoot length (cm), average number of shoots/inoculum, fresh weight (FW) of shoots per explant (mg), dry weight (DW) and water content (WC). As a note, to obtain dry weight (DW), the plant material was dried for three days at 45°C and reweighed. The water content expressed as a percentage (WC%) was calculated using the formula published by Mazurek et al., 2021:

WC (%) = ((Fresh Weight - Dry Weight)/Fresh Weight) * 100

Biochemical analyses

Photosynthetic pigments

The content of Chlorophyll a (Chl a), Chlorophyll b (Chl b) and Carotenoids (Caro) were determined via spectrophotometry, using fresh plant material from in vitro grown shoots (50 mg). The samples were weighed and homogenized and extracted with 90% acetone in water until the residue was colorless. Absorbance was read at 645, 663, and 450 nm using a Perkin Elmer Lambda 25 spectrophotometer. The following formulas were used to quantify the Chl a and Chl b:

Chl a mg/g FW = (11.75 × A663 – 2:35 × A645) × V/g

Chl b mg/g FW = (18.61 × A645 – 3.96 × A663) × V/g

where: A645 and A663 represent the optical density at a specific wavelength, V represents the volume of the extract (mL), and g represents sample weight (mg).

The concentration of total Caro was calculated according to the formula used by Britton et al., 1995:

X mg carotenoids = (A × V × 1000) / (A 1% 1 cm × 100),

where: A represents absorbance at 450 nm, V represents volume (mL), and A1% 1 cm = 2500 and expressed as mg Caro/g fresh material.

Antioxidant compounds

Preparation of methanolic extract: 0.25 g fresh plant material (*in vitro* grown shoots) was

prepared in 2.5 ml methanol, vortexed for 30 sec, then sonicated for 15 min and centrifuged for 10 min at 10000 rot/min at room temperature. The obtained supernatant was filtered through a 0.45 µ nylon filter.

Total phenolic compounds (TPC). The content of TPC was determined by the Folin-Ciocalteu method (Dulf et al., 2015) using a Perkin Elmer Lambda 25 spectrophotometer. The reaction mixtures (200 µl sample, 250 µl Folin-Ciocalteu reagent, 700 µl Na_2CO_3 solution, and 2700 µl water) were incubated at room temperature for 45 min in the dark. After that, the absorbance was measured at 750 nm. Gallic acid (GA) was used as the standard. TPC contents were expressed as mg eq. GA g^{-1} FW.

Total flavonoid content (TFC). TFC was determined using the method developed by Zhishen et al., (1999). Briefly, 500 µl methanol extract was mixed with 200 µl $NaNO_2$ (5%); after 5 min, 200 µl $AlCl_3$ (10%) was added. After 5 min standing, 1500 µL NaOH (1M) and 1100 µl water were added and the absorbance was measured after 15 min, at 510 nm with methanol as blank and the TF contents were expressed as equivalents of the standard Quercetin (mg eq. Q g^{-1} FW)

Quantification of osmolytes.

Proline (Pro): 0.1 g fresh ground shoot material was homogenized in 3% (w/v) sulphosalicylic acid and centrifugated at 3000 rpm for 10 min; 2 mL of the supernatant was mixed with 2 mL acid ninhydrin, incubated in a water bath at 100°C for 1 h, chilled on ice, and then extracted with 4 mL toluene. The proline concentration was determined from a standard curve calculated in fresh weight according to Bates et al. (1973).

The absorbance was measured at 520 nm using toluene for blank, and Pro concentrations were expressed as µmol g^{-1} FW.

Oxidative stress markers

Malondialdehyde (MDA): 0.2 g of fresh plants were mixed with 3 mL of 0.1% trichloroacetic acid (TCA), mixed for 30 s followed by centrifugation at 10,000 rpm, 4°C for 10 min. An aliquot of 0.5 mL supernatant was transferred into another tube and mixed with 1.5 mL of 0.5% thiobarbituric acid (TBA) prepared in 20% TCA. The samples were incubated for 30 min at 95°C in a constant temperature water bath and then cooled in an ice bath for 5 min. After centrifugation at 10,000 rpm for 10 min at 4°C, the absorbance of the obtained supernatant was detected at 532, 600, and 450 nm using a Lambda 25 (Perkin-Elmer Singapore) UV-Vis spectrophotometer. MDA concentrations were determined using the equation: $MDA = 6.45(A_{532}-A_{600})-0.56A_{450}$

MDA concentrations were calculated in nmol g^{-1} FW (Hodges et al., 1999).

Hydrogen peroxide (H_2O_2). For H_2O_2 quantification 0.1 g of fresh shoots were homogenized in an ice bath with 5mL of 0.1% (w/v) TCA and the extract was centrifuged at 12000 g for 15 min. 0.5 mL of the supernatant was combined with 0.5 mL of 10 mM potassium phosphate buffer (pH = 7) and 1 mL of 1M potassium iodide (KI) from Duchefa Biochemie BV (Haarlem, The Netherlands). After incubation for 30 min at room temperature, the absorbance was measured at 390 nm. The content of H_2O_2 was calculated by comparison with a standard calibration curve using different concentrations of H_2O_2.

Antioxidant activity

DPPH free-radical-scavenging activity was performed using the method described by Brand-Williams et al. (1995) with slight modifications. First, 35 µL of each leaf methanolic extract was mixed with 250 µL of freshly prepared DPPH solution (80 µM in methanol). The absorbance was measured after 30 min of standing, under dark conditions, at 515 nm, using a Synergy HT microplate reader (BioTeK®, USA) and methanol as blank. In the DPPH assay, the antioxidant activity of the extracts was evaluated using the calibration curve performed with Trolox, and then the absorbance was recorded for all the tested extracts, to calculate the percentage inhibition (expressed as percentage inhibition of the DPPH radicals). The percentage inhibition (I%) was calculated as $I\% = [(AB-AA)/AB]\times100$, where: AB = absorbance of blank and AA = absorbance of methanolic extract.

Statistical analysis

One-way analysis of variance (ANOVA) was performed. Post hoc testing for the ANOVAs was performed using Tukey's honestly significant difference test (Tukey's test) using a $P<0.05$ significance level to determine the statistically significant differences between the

means. Values shown (in text and figures) are means ± SE (standard error).

RESULTS AND DISCUSSIONS

In vitro growth parameters

The growth parameters were measured after ten weeks of *in vitro* culture on WPM medium supplemented with 100 mg/L Sequestrene and 0.5 mg/L Z in various PEG 6000 treatments. These treatments included 0 g/L, 10 g/L, 20 g/L, 30 g/L, 40 g/L, and 50 g/L PEG 6000 (Figure 1). The measured parameters included the number of proliferated shoots per initial inoculum, shoot length, fresh weight, and dry weight per proliferated inoculum, as well as the water content (Table 1). As observed in Table 1, hydric stress resulted in an increase in the number of shoots per inoculum in all applied PEG treatments. The highest number of proliferated shoots was obtained when the culture medium was treated with 20 g/L PEG (17.09±0.52) and decreased with the increasing concentration of PEG, reaching 13.21±0.76 under the treatment with 50 g/L PEG. Contrary to the number of proliferated shoots, their length decreased with the increasing concentration of PEG, such that in the treatment with 50 g/L PEG, the length of the shoots was halved (Table 1).

In all PEG treatments, there was an increase in fresh weight compared to the control without PEG. The largest increase was observed in the treatment with 40 g/l PEG (170.43±5.40 mg), which was 53% higher than in the culture medium without PEG (Table 2).

In terms of shoot water content, it increased with the rising PEG concentration. Shoots grown without PEG showed the lowest water percentage (75.44%), while it increased by 11.7% in shoots grown in a medium treated with 50 g/l PEG (Table 2).

Therefore, among the morphological parameters, shoot length was strongly affected by drought stress at all PEG concentrations (Table 1). Growth inhibition is a common response of plants subjected to water stress and has been reported for various plant species cultivated in vitro on culture media under different PEG concentrations. Thus, similar to blueberry, water stress reduced shoot length in *Vanilla planifolia* (Martínez-Santos et al., 2021), *Saccharum* spp. Hybrids (Hernández-Pérez et al., 2021), *Viola odorata* (Darvishani et al., 2020), and *Prunus avium* (Vuksanović et al., 2022). Water stress induced by PEG also had a negative impact on shoot length of *Myrtus communis* (Tafreshi et al.,2021).

In present study, a significant increase in the number of proliferated shoots/initial inoculum as well as in fresh weight was observed under all concentrations of PEG. Few previous reports have focused on the increase in the number of proliferated shoots under drought stress conditions. This may be due to the fact that PEG-supplemented culture media did not contain growth regulators. Contrary to our findings, Vuksanović et al. (2022) reported a lower number of proliferated shoots under drought stress induced by PEG (20 and 50 g/l) compared to the culture medium without PEG (MS supplemented with 1 mg/l BA and 0.5 mg/l kinetin) for two clones of Wild cherry. Also, Tafreshi et al. (2021) show that the total number of proliferated shoots of *M. communis* was negatively affected by the content of PEG in the culture medium. Shoot number was reduced for explants grown in media containing 3% PEG and 6% PEG compared to control explants without PEG.

However, *Stevia rebaudiana* behaved similarly under drought stress conditions induced by PEG and had the highest average number of nodes, leaf number, and the highest fresh weight of shoots under the 4% PEG treatment followed by the results obtained under 2%, 1%, and 0.5% PEG 6000 treatments (Ahmad et al., 2020).

Biochemical analysis

Significant differences were observed in the contents of Chl a, Chl b, and Caro in *V. corymbosum* shoots exposed to different concentrations of PEG 6000. The highest contents of Chl a (1.48±0.06 mg/g FW), Chl b (0.64±0.03 mg/g FW), and total Caro (0.88±0.062 mg/g FW) were recorded in shoots grown in the culture medium without PEG. The lowest contents of photosynthetic pigments were recorded on culture media with 50 g/l PEG, with the general tendency being a decrease in the concentration of photosynthetic pigments with increasing PEG concentration (Table 2). The content of photosynthetic pigments is an important indicator of plant physiological status

and diminished levels of these pigments have often been linked to drought conditions (Vuksanović et al., 2022). The decrease in Chl a and Chl b content in blueberry shoots, due to water stress, induced by the five concentrations of PEG in our study aligns with findings from various other studies conducted on different species. For example, Martínez-Santos et al. (2021) observed reduced levels of Chl a, Chl b, and total chlorophyll in *Vanilla planifolia* under drought stress induced by 1%, 2%, and 3% PEG. Similarly, Gao et al. (2020) documented the inhibitory effect of drought on the concentrations of Chl a and Chl b in *Dendrobium officinale* across different drought stress conditions (10, 30, and 50 g/l PEG). This decrease in photosynthetic activity under stress conditions can be attributed to stomatal closure, which is also a response mechanism to avoid energy waste (Martínez-Santos et al., 2021).

Figure 1. Effect of different concentrations of polyethylene glycol (PEG 6000) on the growth of the *in vitro* culture of highbush blueberry, cv Hortblue Petite: (a) 0 g/L PEG 6000; (b) 10 g/L PEG 6000; (c) 20 g/L PEG 6000; (d) 30 g/L PEG 6000; (e) 40 g/L PEG 6000; (f) 50 g/L PEG 6000

Table 1. Growth parameters measured after ten weeks of *in vitro* drought stress treatments in the Hortblue Petit highbush blueberry variety. Values are presented as means ± SE. Different lowercase letters indicate significant differences between treatments for each determined variable according to the Tukey test ($p < 0.05$)

Treatments	Parameters									
	No. of shoots/ inoculum	% of reduct ion*	Length of shoots (cm)	% of reduc tion	Fresh weights (mg)	% of reduct ion	Dry weights (mg)	% of reduct ion	Water content %	% of reduct ion
0 PEG	8.80±0.56 a	0	1.99±0.56 c	0	110.23±3.25 a	0	25.03±4.32 ab	0	75.44±3.39 a	0
10 g/l PEG	16.27±1.00cd	-84.85	1.49±1.00 b	25.10	141.93±6.49 b	-28.76	27.20±1.87 c	-8.66	79.88±1.32 b	-5.89
20 g/l PEG	17.09±0.52 d	-94.19	1.08±0.52 a	45.62	147.10±6.82 b	-33.44	26.17±1.98 bc	-4.53	81.64±0.82 c	-8.21
30 g/l PEG	15.69±0.42 c	-78.28	0.96±0.42 a	51.79	118.13±4.47 a	-7.17	23.43±5.64 a	6.39	80.87±2.13 bc	-7.20
40 g/l PEG	16.78±1.11 d	-90.66	0.98±1.11 a	50.86	170.43±5.40 b	-54.61	28.87±7.83 d	-15.26	81.28±1.06 c	-7.74
50 g/l PEG	13.21±0.76 b	-50.09	0.99±0.76 a	50.00	149.87±3.23 b	-35.95	24.20±5.92 a	3.33	84.04±1.00 d	-11.40

*% - Percentages of reduction - the control average (0 PEG) was considered 100%)

Table 2. Effect of *in vitro* drought stress in the Hortblue Petit highbush blueberry shoots on contents of photosynthetic pigments (chlorophyll a - Chl A, chlorophyll b - Chl b, carotenoids - Caro), proline (Pro), malondialdehyde (MDA), hydrogen peroxide (H_2O_2)

Treatments	Biochemical analysis					
	Chl A (mg/g FW)	Chl B (mg/g FW)	Caro (mg/g FW)	Pro (µmole proline/g FW)	MDA (nmol/g FW)	H_2O_2 (µmol/g FW)
0 PEG	1.48±0.06 e*	0.64±0.032 d	0.88±0.062 d	4.59±0.109 a	339.45±0.001 a	236.0±0.003 a
10 g/l PEG	0.63±0.06 b	0.45±0.010 c	0.41±0.020 ab	4.95±0.034 b	473.21±0.012 b	373.3±0.044 b
20 g/l PEG	0.86±0.01 d	0.32±0.011 b	0.53±0.011 c	5.23±0.123 c	502.88±0.001 bc	481.9±0.075 c
30 g/l PEG	0.80±0.01 c	0.31±0.012 b	0.50±0.042 bc	6.50±0.041 d	519.17±0.001 bc	514.5±0.031 d
40 g/l PEG	0.63±0.03 b	0.24±0.033 a	0.41±0.033 ab	6.92±0.034 e	558.10±0.001 c	577.5±0.021 e
50 g/l PEG	0.55±0.06 a	0.21±0.028 a	0.36±0.058 a	7.69±0.507 f	835.75±0.001d	599.7±0.007 e

*Values are presented as means ± SE. Different lowercase letters indicate significant differences between treatments for each determined variable according to the Tukey test ($p < 0.05$

As shown in Table 2, Pro accumulation was significantly higher in plants stressed by drought compared to those grown in the culture medium without PEG. The highest amount of proline (7.69 µmole proline/g FW) accumulated in the shoots grown on the culture medium with 50 g/l PEG, which was 1.68 times higher than the control.

Furthermore, a positive correlation can be observed between Pro accumulation and increasing drought levels. The highest Pro accumulation was recorded at maximum drought levels, similar to observations in other species subjected to drought stress induced by PEG (Piwowarczyk et al., 2014; Razavizadeh et al, 2019; Jiroutova et al., 2021). However, the proline content of *Myrtus communis* shoot significantly increased 5.7-fold under 3% PEG treatment compared to the control without PEG. In contrast, in the 6% PEG treatment, proline content was considerably lower compared to the 3% PEG treatment and was not statistically different from the control (Tafreshi et al., 2021). These results suggest that Pro has a role in plant drought stress tolerance mechanisms and the accumulation of Pro in plants is a physiological response to drought stress manifested by reduced plant growth.

Oxidative stress markers, malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) exhibited a significant increase in shoots grown on culture media treated with all concentrations of PEG compared to culture media without PEG (Table 2). MDA reached its highest value on the culture medium with 50 g/l PEG (835.75±0.001 nmol/g FW), which was twice as high as the control without PEG (339.45±0.001 nmol/g FW). A similar response to drought stress was observed for H_2O_2, with concentrations of 599.7±0.007 µmol/g FW in shoots grown on culture medium supplemented with 50 g/l PEG, compared to 236.0±0.003 µmol/g FW in shoots of blueberries grown on culture medium without PEG. The results of this study are consistent with those reported in previous studies. For example, MDA content in *Vigna mungo* plants increased to the maximum level with increasing severe concentrations of PEG where a 20% increase was observed compared to the control (Jothimani & Arulbalachandran, 2020). Similarly, osmotic stress significantly increased MDA content relative to the control in the cucurbit species, and the highest MDA content was observed under the highest concentration of PEG (Tajaragh et al., 2022). Additionally, the highest content of H_2O_2 was obtained under the highest concentration of PEG added to the

culture medium for the three Iranian *Cucurbita* sp. (Tajaragh et al., 2022), which is in accordance with our results.

In the case of total flavonoids (TF) and total phenolic content (TPC), Table 3 demonstrates that the highest levels (TPC 9.07±0.044 mg eq. GA/g FW and TF 4.67±0.022 mg Q/g FW) were observed in blueberry shoots grown in culture medium supplemented with 50 g/l PEG 6000. Conversely, the lowest levels of antioxidant activities (TPC 8.26±0.101 mg eq. GA/g FW and TF 3.69±0.005 mg Q/g FW) were recorded in blueberry shoots grown in the medium containing 10 g/l PEG 6000. In general, TPC and TFC content and DPPH in the blueberry shoots were reduced at mild stress and increased at intense osmotic stress conditions. The highest level in both traits was obtained in the shoots treated with 50 g/l PEG (Table 3).

The accumulation of secondary metabolites, such as total flavonoids (TF) and total phenolic content (TPC), is crucial for plant adaptation to environmental stressors and serves a vital role in protection against free radicals. Previous studies have shown that different plant species exhibit various responses in terms of secondary metabolite accumulation under stress conditions.

Table 3.Total phenolic content (TPC), total flavonoid content (TF), and DPPH activity of the Hortblue Petit highbush blueberry shoots under *in vitro* drought stress

Treatments	TF (mg Q/g FW)	TPC (mg eq. GA/g FW)	DPPH (I%)
0 PEG	4.56±0.083 b	8.93±0.098 b	92.42
10 g/l PEG	3.69±0.005 a	8.26±0.101 a	86.27
20 g/l PEG	3.70±0.192 a	8.37±0.552 a	88.37
30 g/l PEG	4.52±0.028 b	8.66±0.443 a	77.12
40 g/l PEG	4.54±0.114 b	8.98±0.016 b	75.42
50 g/l PEG	4.67±0.022 b	9.07±0.044 c	87.45

*Values are presented as means ± SE. Different lowercase letters indicate significant differences between treatments for each determined variable according to the Tukey test ($p < 0.05$).

For instance, Vuksanović et al. (2022) observed lower levels of TF and TPC in *Prunus avium* grown in vitro under stress conditions compared to the control without PEG. Similarly, Puente-Garza et al. (2017) reported a decrease in TF content under drought stress in *Agave salmiana*. As in the case of blueberry, *Stevia rebaudiana* had the highest content in TPC, TFC, TAC, TRP, and DPPH-FRSA, (TPC 15.03 µg/mg, TFC 13.01 µg/mg, TAC 22.26 µg /mg, TRP 19.87 pg/mg and DPPH-FRSA 95.27%) in shoots grown in MS medium supplemented with 4% PEG 6000 stress. It was followed by the amounts obtained under 2% PEG 6000 stress and later, 1% PEG 6000 stress, followed by 0.5% PEG 6000 stress. The lowest amount of antioxidant activities (TPC 6.97 µg/mg, TFC 4.44 µg/mg, TAC 13.7 µg/mg, TRP 10.22 µg/mg, and DPPH-FRSA 48.03%) was elucidated by shoots grown in PEG60-deficient medium (Ahmad et al., 2020).

CONCLUSIONS

In vitro drought stress induced by PEG 6000 had effects on growth and biochemical determinations evaluated in *V. corymbosum*. The most significant parameters that responded to drought stress in our study were: shoot length, shoot number, content of photosynthetic pigments, proline, malondialdehyde, and hydrogen peroxide. A reduction in proliferated shoot length and an increase in shoot number were observed on the WPM + 1 mg/l Z culture medium supplemented with 10, 20, 30, 40, and 50 g/l PEG. The content of photosynthetic pigments decreased, while the content of proline, malondialdehyde, and hydrogen peroxide increased with the increasing concentration of PEG 6000 in the culture medium. The results obtained suggest that the in vitro screening technique of highbush blueberries for drought tolerance, utilizing PEG as a stress agent, could serve as an alternative method for the early selection of drought-resistant cultivars. However, for validation, field research is recommended in line with practices for other species.

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