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Improvement of Morphological and Biochemical Traits of *In vitro Antigonon leptopus* Plant Grown under Drought Conditions

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Abstract

The micropropagation ability of *Antigonon leptopus* plant under drought stress was examined using three levels (0, 1, 2%) of polyethylene glycol (PEG) with additive two concentrations (1 and 2gl^{-1}) of potassium silicate (K_2SiO_3) as well as silica nanoparticles (SiO_2-NPs) at 2.5 and 5 mgl^{-1} as protectants to mitigate drought stress. Most treatments of the two sources of silicate enhanced the ability of explants to survive highly as compared to those grown under drought condition only. Adding 1gl^{-1} of K_2SiO_3 or 2.5mgl^{-1} of SiO_2-NPs positively affected the morphological characters and all estimated pigments content under various levels of PEG (0, 1 and 2%) in the culture medium. High level (2%) of PEG without any additive augmented the phenolic compounds (Tannins, flavonoids and phenols) as well as proline and catalase activity. The total soluble sugar was increased under high levels of PEG (2%) and/or K_2SiO_3 (2gl^{-1}) individually or interacted as compared with other treatments. The fractionation of phenolic compounds by HPLC showed an increment in some components at 2% PEG such as apigenin, cinnamic acid and ellagic acid compared to the control.

Keyword: Antigonon leptopus, drought, potassium silicate, silica nanoparticles, and phenolic compounds

1. Introduction

Antigonon leptopus is a fast-growing vine with heart-shaped leaves, belonging to the Polygonaceae family and native to Mexico. It has amazing, bright pink flowers that are borne in clusters. The plant is grown as an ornamental plant for its beautiful flowers [1]. It is an evergreen woody climber vine, distributed in different parts of the world. It possesses various bioactivities such as antioxidant, antimicrobial, hepatoprotective, anti-inflammatory, analgesic and antidiabetic activities [2].

Drought stress is one of the most serious abiotic stresses on crop productivity. Lack of adequate water for normal growth disrupts cell division, root cell proliferation, leaf surface and stem growth, causing raises ROS levels and negative impacts on plant growth [3]. Plant physiology is greatly affected by abiotic stresses. Climate change is known to activate the influence of abiotic stress, particularly drought on plant performance where, drought leads to water deficiency, nutrient imbalance, ionic toxicity and the appearance of oxidative stress [4]. Drought stress has been proven in various studies to impact plants' morphological, physiological, biochemical, and molecular features [5]. Drought also disrupts the flow of electrons, which damages the photosystem by oxidative stress and slowing down photosynthesis [6].

Induction of drought stress is the most widely used method, which uses high molecular weight osmotic agents such as polyethylene glycol (PEG) [7]. This compound did not show any harmful effects on the plants [8]; however, it restrict the development of plants by reducing the water potential of the culture medium, such as soil drying and preventing cultured

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explants from water absorbing [9]. To face the negative effects of water deficiency in plants, several strategies have been proposed. Among these strategies, applications of antitranspirants are used to decrease the rate of transpiration and reduce the harmful effects of drought stress [10].

Silicon (Si) is a useful element for plants, where it can mitigate the negative effects of various environmental restrictions [11]. It plays an important role in drought mitigation, as it greatly increases the water-holding capacity of soil [12]. Si application has been considered as an environmentally friendly practice to increase the production of crops [13] and has become an important strategy for preventing various stresses [14]. The growth and morphogenesis *in vitro* cultures of plants are significantly influenced by the culture medium composition. The addition of Si to the tissue culture medium improves embryogenesis,organogenesis, growth characteristics, and enhances abiotic stress tolerance [15].

To maximize the absorption of silicon and improve the plant's physiological response, new sources and concentrations of silicon are being evaluated. Although potassium silicate is the major source of silicon that is supplied to plants, new sources have recently appeared as an alternative to silicate in plants' nutrition such as nano-silica. However, there is a shortage of studies showing the positive impact of these sources on growth plants [16]. Therefore, the aim of the present work was to assess the efficacy of two sources of silicon (K_2SiO_3 and SiO_2-NPs) to mitigate the adverse effects of drought stress on *Antigonon leptopus* growth *in vitro.*

2. Materials and Methods

The present study was conducted at the Tissue Culture Technique Laboratory, Department of Ornamental Plants and Woody Trees, Central Laboratories, National Research Centre (NRC), Egypt, during the years 2022 and 2023**.**

2.1. Procedure layout

Plant material and surface sterilization

Antigonon leptopus (Corallita) stem nodel explants (3-5 cm length) were collected from a climbing vine maintained at the Zohrya Botanical Garden, Zamalek- Cairo, Egypt. The nodal segments were rinsed in septol soap with shaking for 20 min., then washed with running tap water for one hour and rinsed three times in sterile demineralized water. In a laminar air-flow hood, under aseptic conditions, the explants were dipped for 30 sec in 70% (v/v) ethanol solution (C_2H_5OH) followed by 15% (v/v) commercial sodium hypochlorite solution (NaOCl 5.25%) and one drop of tween 20 ($C_{58}H_{114}O_{26}$) with shaking for 5 min then rinsed three times with autoclaved distilled water. Finally, the explants surface was immersed in mercuric chloride (Hg_2Cl) at 0.1% then for four times, the explants were rinsed with sterile distilled water.

Culture medium and incubation condition

The sterilized explants were cultured initially on a basal MS medium free of hormones at full salt strength for one month. The aseptic culture was subcultured on MS medium supplemented with 2ip at 0.2 mgl^{-1} + IBA at 0.1 mgl^{-1} + 25 gl^{-1} sucrose + 7 gl^{-1} agar for *in vitro* shootlets proliferation, MS described by Youssef *et al*. 2021 on Antigonon leptopus. For testing the *in vitro* micropropagation ability under drought stress, media included three levels of water stress agent {polyethylene glycol (PEG)} (0, 1, 2%) with additive treatments of potassium silicate (K_2SiO_3) at 1 and 2 gl^{-1} as well as silica nanoparticles (SiO_2-NP_s) at 2.5 and 5 mgl^{-1} to mitigate drought stress. On a growth chamber, cultures were incubated at 25±2^{o}C under photoperiod 16h of fluorescent light with 30µmol m^2sec^{-1}.

data were recorded after two months represented in survived explants (%), shootlets length (mm), shootlets number/explant, leaves number /shootlet, and the *in vitro* rooting parameters (rooting %, roots number/shootlets and root length mm).

Silica NPs' characterizations

The silica nanoparticles (SiO_2-NP_s) used in this investigation were acquired from Sigma Corporation in the United States and were examined using an electron microscope. According to Zafar *et al*. (2016) [17], NPs are suspended in consistently distilled and diffused water. Fig. (1) provides illustrations of the Silica NPs' specification.

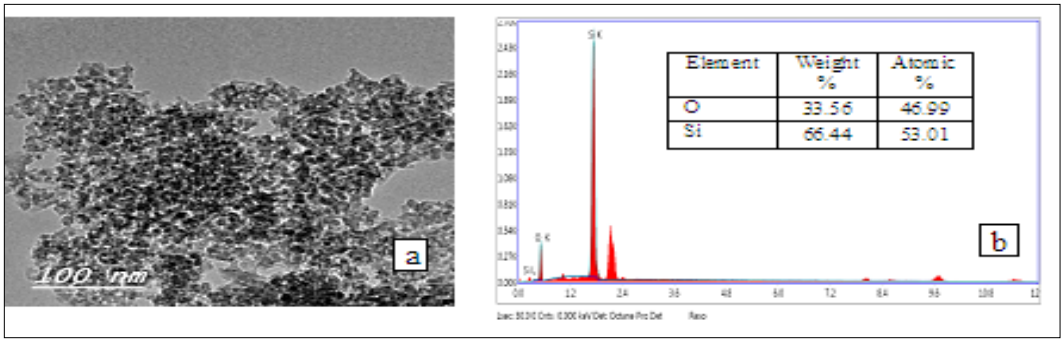

Fig.1. (a): Silica nanoparticles characterization using Scanning electron microscopy (SEM), (b): The chemical composition of the SiO_2-NPs was determined using an X-ray detector and energy dispersive X-ray spectroscopy. The elemental proportions of Si and O are shown in the image inset

2.2. Biochemical analysis

Photosynthetic pigments: chlorophyll a and b as well as total carotenoids were determined according to Saric *et al.* (1982). [18]

Anthocyanin pigment: Anthocyanin was extracted overnight from fresh shootlets with ethanol and 1% HCl (85:15) at 4°C. The optical density of the extract solution was measured at 535 nm. according to Francis (1982) [19].

Shootlets extraction: *Antigonon leptopus* shootlets (5g) were soaked in ethanol 50 ml (80 %) and shaken for 48 h at room temperature. The extracts were filtered and extracted twice.

Phenolic compounds: For the assay of the phenolic compound, the final extract was used. Total phenol was assayed using Folin–Ciocalteu's reagent according to Singleton and Rossi, (1965) [20]. The tannins content was determined according to Tambe *et al.* (2014) [21]. Total flavonoid was assayed by the method of Zhishen *et al.* (1999) [22].

Identification of phenolic compounds by using HPLC: HPLC analysis (C18: (4.6 mm ID x 250 mm, 5 µm) was performed using an Agilent 1260 series. The mobile phase consisted of water (A) and 0.05% trifluoroacetic acid in acetonitrile (B) at a flow rate of 0.9 ml/min. The mobile phase was programmed sequentially with a linear gradient as follows: 0 min (82% A); 0–5 min (80% A); 5-8 min (60% A); 8-12 min (60% A); 12-15 min (82% A); 15-16 min (82% A) and 16-20 (82%A). The multi-wavelength detector was monitored at 280 nm. The injection volume was 5 µl for each of the sample solutions. The column temperature was maintained at 40 °C.

Determination of proline content: Proline content was measured in dried leaves using the method determined by Bates *et al*. (1973) [23].

Total sugar: Total sugar was estimated using ethanol extract, phenol, and sulphuric acid reagent method described by Dubois *et al.* (1956) [24].

Antioxidant enzyme extraction and determination of catalase activity: *Antigonon leptopus* fresh shootlets (0.1g) were grind and then extracted with 5 ml of ice-cold buffer phosphate (PH 7.4). At 10,000 rpm for 30 min, the homogenate was centrifuged and the supernatant was collected. The resulting supernatant was used for the determination of enzyme activity. The catalase activity in the supernatant was determined by the method of Nakano and Asada (1981) [25].

2.3. Statistical analysis

The treatments' means were compared for significance by Duncan's (1955) [26] New Multiple Range test at a 5% level of probability using MSTAT Computer Program (MSTAT Development Team, 1989) [27]. Analysis of variance was arranged as two factorials in a randomized complete block design. The data were

statistically analyzed using analysis of variance according to Steel and Torrie (1980) [28].

3. Results and discussion

3.1. Survival and *in vitro* growth

The effects of potassium silicate (K_2SiO_3) at 0, 1 and 2 mg/l as well as silica nanoparticles (SiO_2-NPs) at 0, 2.5 and 5 mg/l on survived and grown *Antigonon leptopus* plant under various drought levels (0, 1 and 2%) were illustrated in Table (1). The survived explants could be obtained at 100% with all treatments of the two sources of silicate except for 2.5 mgl^{-1} of SiO_2-NPs which lowered this percent to 95.67% comparing to control which caused the lowest one (88.89%). The negative effect of drought stress was also noticed on survived explants which were decreased with increasing PEG concentration in the culture medium. The interaction effect of drought conditions and the different concentrations of most used protectants enhanced the ability of explants to highly survive as compared to those grown under drought condition only.

Table 1. Effect of potassium silicate (K_2SiO_3) and silica nanoparticles (SiO_2-NPs) on survival, shooting and rooting of *in vitro Antigonon leptopus* plant grown under drought conditions

Treatment A	Treatment B	Survival %	Shoot length	Shoot number	Leaves number	Rooting %	Root number	Root length
0% PEG	Control	100 A	87.33B	1.67 CDE	18.00 CDEF	71.67 B	2.33 BC	30.33 DE
	$1gl^{-1}$ K_2SiO_3	100A	91.67A	3.33 A	27.67 A	88.67 A	4.33 A	54.33 A
	$2gl^{-1}$ K_2SiO_3	100A	84.67B	2.67 ABC	20.67 BC	77.33 AB	3.00 B	45.00B
	$2.5mgl^{-1}$ SiO_2-NPs	100A	87.67B	3.00 AB	23.33 B	83.00 AB	3.00 B	36.00 C
	$5mgl^{-1}$ SiO_2-NPs	100A	80.33C	2.00 BCDE	19.33 CD	72.00 B	2.67B	32.33CD
1% PEG	Control	88.67B	36.67H	1.33 DE	12.33 HIJ	0.00 D	0.00D	0.00G
	$1gl^{-1}$ K_2SiO_3	100A	55.67D	2.67 ABC	18.67 CDE	71.67 B	2.33BC	26.67E
	$2gl^{-1}$ K_2SiO_3	100A	49.67E	1.67 CDE	15.00 FGH	0.00 D	0.00D	0.00G
	$2.5mgl^{-1}$ SiO_2-NPs	100A	51.67E	2.33 ABCD	17.00 DEFG	0.00D	0.00D	0.00G
	$5mgl^{-1}$ SiO_2-NPs	100A	44.67F	1.67 CDE	12.33 HIJ	0.00D	0.00D	0.00G
2% PEG	Control	78.00C	32.33I	1.00 E	9.67 J	0.00D	0.00D	0.00G
	$1gl^{-1}$ K_2SiO_3	100A	42.67 FG	2.00 BCDE	16.00 EFG	55.33C	1.67C	18.33F
	$2gl^{-1}$ K_2SiO_3	100A	39.67 GH	1.33 DE	11.67 IJ	0.00D	0.00D	0.00G
	$2.5mgl^{-1}$ SiO_2-NPs	87.00B	40.00 GH	1.67 CDE	14.00 GHI	0.00D	0.00D	0.00G
	$5mgl^{-1}$ SiO_2-NPs	100A	37.33 H	1.33 DE	11.33 IJ	0.00D	0.00D	0.00G
Mean	0%PEG	100A	86.33 A	2.53 A	21.80 A	78.53A	3.07A	39.60A
	1%PEG	97.73B	47.67 B	1.93 B	15.07 B	14.33B	0.47B	5.33B
	2%PEG	93.00C	38.40 C	1.47 B	12.53 C	11.07B	0.33B	3.67B
Mean	Control	88.89C	52.11 D	1.33 D	13.33 D	23.89B	0.78B	10.11C
	$1gl^{-1}$ K_2SiO_3	100A	63.33 A	2.67 A	20.78 A	71.89A	2.78A	33.11A
	$2gl^{-1}$ K_2SiO_3	100A	58.00 B	1.89 BC	15.78 C	25.78B	1.00B	15.00B
	$2.5mgl^{-1}$ SiO_2-NPs	95.67B	59.78 B	2.33 AB	18.11 B	27.67B	1.00B	12.00C
	$5mgl^{-1}$ SiO_2-NPs	100A	54.11 C	1.67 CD	14.33 CD	24.00B	0.89B	10.78C

Control: MS+2ip at $0.2mgl^{-1}$ + IBA at $0.1mgl^{-1}$. [Means within a column having the same letters are not significantly different according to Duncan's Multiple Range Test (DMRT) at 5% level].

In vitro shooting (shoot length and number and leaves number) and rooting (root%, root number and length) of the grown shootlets under drought conditions behave the same trend of survived explants. Increasing PEG concentrations in culture medium reduced the values of these parameters. Meanwhile, the clear positive effects of 1gl^{-1} K_2SiO_3 on these morphological characters were noticed separately as well as when interacted with various levels of PEG (0, 1 and 2%) in the culture medium comparing with other treatments. This positive effect was also indicated on successful acclimatized plants when transferred to the greenhouse (Fig.2). Similarly, a negative impact of drought stress was also recorded on shoot length, shoots number and leaves number of banana plant after adding PEG to MS media [29]. This inhibition effect might be attributed to the slowing down of cell division and/or changes in cell cycle progression [30]. To mitigate these negative effects, one of the best ways to help plants that are suffering from drought stress is exogenous Si therapy [31]. All morphological and biochemical traits were improved on culture media supplemented with K_2SiO_3, either individual or in combination with PEG [32].

A critical role of Si for plant species to mount a defense to numerous stresses (drought, salts, etc.) was mentioned by Ma and Yamaji (2006) [33]. Si is accumulated in plant tissues and cell wall apoplasts for the formation of silica, which serves as tissue protection and a barrier against stressor penetration. The water loss inhibition from the leaves of plants by preserving the plant's hydration status might be one of the processes accountable for outstanding development in the presence of Si under stress conditions [34]. Gong *et al.* 2003 [35] mentioned to that there are noticed reduction in the transpiration of Si-treated plants, which attributed to the formation of the pair layer of silica cuticle in leaf epidermal tissue.

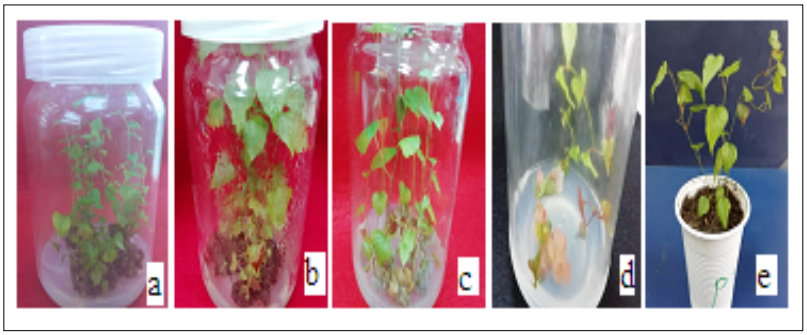

Fig. 2 (a-e): *In vitro* shooting and rooting ability of *Antigonon leptopus* (a) Shootlets development that were cultured on MS+2ip at 0.2 mgl^{-1} +IBA at 0.1mgl^{-1} (The control), (b) Shootlets treated with potassium silicate, (c) Shootlets grown under drought conditions at 1%PEG, (d) Shootlets grown under drought conditions at 2%PEG, (e) Acclimatized *Antigonon leptopus* plant.

3.2 Photosynthetic pigments and anthocyanin pigment

Drought conditions decreased the estimated pigments contents (Chl. a, b and carotenoid) to obtain the lowest values (56.70, 23.07 and 26.63 mg/100gm F.W., respectively) with 2% PEG, in comparison with those of control (Table 2). Meanwhile, the highest anthocyanin content (25.03 mg/100g F.W.) was recorded for the obtained shootlets treated with the highest PEG concentration (2%) in the culture medium.

The treatment of K_2SiO_3 at 1g/l increased these pigments to the highest values (73.25, 30.89, 34.61 and 23.83 mg/100 g F.W., respectively). Under

drought condition (1 or 2% PEG), the applications of 1gl^{-1} of K_2SiO_3 improved all pigments content as compared to other treatments.

Confirmed study by Mahmoud *et al*. 2020 [36] mentioned that the content of chlorophylls in the leaves was decreased due to the drought. The reduced photosynthetic pigments under drought condition might be due to that long-term dryness reduces mesophyll conductance, which lowers CO_2 diffusion and lowers photosynthesis in leaf tissue [37].

Anthocyanin performs as osmoregulatory to preserve water homeostasis, so under stress of drought, plants transcriptionally organize genes of anthocyanin biosynthesis to enhance the production of anthocyanin pigment [38]. Anthocyanin probably scavenge the drought stress-induced ROS that enter the vacuoles, preventing chain reactions to prevent too much ROS accumulation and preserving water homeostasis for plant growth and drought stress tolerance [39]. Ma *et al.* (2015) [40] reported that silicon, either in bulk form or as a nanoparticle formulation, can enhance the photosynthetic apparatus even under the negative effects of abiotic stress.

Table 2. Effect of potassium silicate (K_2SiO_3) and silica nanoparticles (SiO_2-NPs) on photosynthetic and anthocyanin pigments (mg100g^{-1} F.W.) of *in vitro Antigonon leptopus* plant grown under drought conditions

Treatment A	Treatment B	Chlorophyll- a	Chlorophyll-b	Total carotenoid	Anthocyanin
0% PEG	Control	71.66 BC	26.07 DEF	31.11 DEFG	17.79 G
	1gl^{-1} K_2SiO_3	80.22A	33.45A	38.24 A	20.37F
	2gl^{-1} K_2SiO_3	72.60B	29.81 ABCD	34.51 BCD	18.68G
	2.5mgl^{-1} SiO_2-NPs	75.25B	31.41 AB	36.32 AB	18.17G
	5mgl^{-1} SiO_2-NPs	71.94BC	27.84 BCDE	32.53 CDE	17.96G
1% PEG	Control	60.63 FG	23.84 EFG	28.68 FGH	22.48D
	1gl^{-1} K_2SiO_3	73.51 B	31.83 A	34.76 BC	23.58C
	2gl^{-1} K_2SiO_3	64.82 E	27.79 BCDE	31.56 CDEF	21.43E
	2.5mgl^{-1} SiO_2-NPs	68.65 CD	30.76 ABC	33.08 BCDE	20.46F
	5mgl^{-1} SiO_2-NPs	63.17 EF	26.34 DEF	29.84 EFG	18.61G
2% PEG	Control	49.29 J	19.63 H	23.24 J	25.29B
	1gl^{-1} K_2SiO_3	66.02 DE	27.39 CDE	30.82 EFG	27.55A
	2gl^{-1} K_2SiO_3	56.14 HI	24.22 EFG	26.08 HIJ	25.81 B
	2.5mgl^{-1} SiO_2-NPs	58.33 GH	23.08 FGH	27.91 GHI	22.95 CD
	5mgl^{-1} SiO_2-NPs	53.70I	21.04GH	25.12 IJ	23.55C
Mean	0%PEG	74.33A	29.72A	34.54A	18.59C
	1%PEG	66.16B	28.11A	31.58B	21.31B
	2%PEG	56.70C	23.07B	26.63C	25.03A
Mean	Control	60.53D	23.18C	27.68D	21.85B
	1gl^{-1} K_2SiO_3	73.25A	30.89A	34.61A	23.83A
	2gl^{-1} K_2SiO_3	64.52C	27.27B	30.72BC	21.97B
	2.5mgl^{-1} SiO_2-NPs	67.41B	28.42 B	32.44 B	20.53 C
	5mgl^{-1} SiO_2-NPs	62.94C	25.07C	29.16CD	20.04D

Control: MS+2ip at 0.2mgl^{-1} + IBA at 0.1mgl^{-1}. [Means within a column having the same letters are not significantly different according to Duncan's Multiple Range Test (DMRT) at 5% level]

3.3 Phenolic compounds

The determined phenolic compounds (Tannins, flavonoids, and phenols) showed a positive response to drought treatments (Table 3). The gradual increment in these compounds was noticed with increasing PEG concentration to 2% which attained to the highest values (17.77 mg g^{-1}, 133.40 mg QEg^{-1} and 102.30 mg $GAEg^{-1}$, respectively). Whereas, the culture medium was supplied with the highest concentrations of potassium silicate ($2gl^{-1}$) or silica NPs ($5\ mgl^{-1}$) led to depression of these compounds as compared to control that obtained the highest values (15.73 mg/g, 124.20 mg QE g^{-1} and 95.35 mg GAE g^{-1}, respectively). The interactive effect showed an increment of the phenolic compounds under the stress treatments, using the highest level of PEG (2%) without any additive treatments of Si resulted in the highest values (22.05 mgg^{-1}, 156.10 mg QEg^{-1} and133.80 mg $GAEg^{-1}$, respectively) followed by the treatment of K_2SiO_3 ($1gl^{-1}$) under 2% PEG level.

These results are in agreement with Mahmoud *et al.* (2020) [36] who demonstrated that increasing Si levels under drought stress caused gradual decrement in the phenolic compounds. Higher amounts of phenolic compounds in the presence of reduced silica levels could imply that silicification is being remediated by the higher phenolic compounds [41].

Table 3. Effect of potassium silicate (K_2SiO_3) and silica nanoparticles (Si O_2-NPs) on tannins, flavonoids and phenols content of *in vitro Antigonon leptopus* plant grown under drought conditions

Treatment A	Treatment B	Tannins (mg/g)	Flavonoids (mg QE/g)	Phenols (mg GAE/g)
0% PEG	Control	7.53I	84.63 M	45.18 O
	$1gl^{-1}$ K_2SiO_3	9.72G	91.07K	55.17 M
	$2gl^{-1}$ K_2SiO_3	8.42H	88.49L	50.04 N
	$2.5mgl^{-1}$ SiO_2-NPs	9.76G	96.16J	61.53 K
	$5mgl^{-1}$ SiO_2-NPs	9.00 GH	91.67K	57.34 L
1% PEG	Control	17.62 C	132.00C	107.10 C
	$1gl^{-1}$ K_2SiO_3	15.05 D	126.20E	91.00 E
	$2gl^{-1}$ K_2SiO_3	12.45E	120.50F	84.75G
	$2.5mgl^{-1}$ SiO_2-NPs	11.90 E	105.70H	78.96I
	$5mgl^{-1}$ SiO_2-NPs	11.06 F	99.98I	74.03J
2% PEG	Control	22.05A	156.10A	133.80A
	$1gl^{-1}$ K_2SiO_3	18.43B	137.60B	110.90B
	$2gl^{-1}$ K_2SiO_3	16.98C	130.60D	97.47D
	$2.5mgl^{-1}$ SiO_2-NPs	17.00C	124.90E	87.82F
	$5mgl^{-1}$ SiO_2-NPs	14.36D	117.70G	81.67H
Mean	0%PEG	8.89C	90.40C	53.85C
	1%PEG	13.62B	116.90B	87.17B
	2%PEG	17.77A	133.40A	102.30A
Mean	Control	15.73A	124.20A	95.35A
	$1gl^{-1}$ K_2SiO_3	14.40B	118.30B	85.69B
	$2gl^{-1}$ K_2SiO_3	12.62C	113.20C	77.42C
	$2.5mgl^{-1}$ SiO_2-NPs	12.89C	108.90D	76.10D
	$5mgl^{-1}$ SiO_2-NPs	11.47D	103.10E	71.01E

Control: MS+2ip at $0.2mgl^{-1}$ + IBA at $0.1mgl^{-1}$. [Means within a column having the same letters are not significantly different according to Duncan's Multiple Range Test (DMRT) at 5% level]

Earlier studies have reported that the addition of silicon to tissue culture media decreases the oxidative phenolic browning and oxidative damage and increases secondary metabolite synthesis, antioxidant enzymes and abiotic stress tolerance [15]. Osmotic adjustment and activation of antioxidative defense system are the key of mechanisms where, silicon mediated drought stress depression in plants is accomplished [42].

3.4. Phenolic profile of *Antigonon leptopus*

Phenolic compounds were identified by HPLC as shown in table (4). When identifying the phenolic compounds, the treatments (2%PEG) with the highest phenolic content was selected and compared to the control. The 2% PEG treatment had the highest content of naringenin (5.49 µg ml^{-1}), apigenin (3.88 µg ml^{-1}), daidzein (1.03 µg ml^{-1}), rutin (0.61 µg ml^{-1}), and ellagic acid (0.15 µg ml^{-1}) compared to the control. Kaisoon *et al*. (2011) [43] mentioned that the main identified phenolic acids in analyzed *Antigonon leptopus* were gallic, and ferulic, while prevailing flavonoids were rutin and quercetin.

Table 4. HPLC phenolic profile of *Antigonon leptopus* extract from selected treatment (2% PEG) compared with the control

Components (µgml^{-1})	Control	Selected treatment (2% PEG)
Gallic acid	0.00	0.00
Chlorogenic acid	0.00	0.00
Catechin	14.57	3.17
Methyl gallate	1.64	0.10
Coffeic acid	0.00	0.00
Syringic acid	0.16	0.14
Pyro catechol	0.00	0.00
Rutin	0.15	0.61
Ellagic acid	0.00	0.15
Coumaric acid	0.09	0.09
Vanillin	0.40	0.42
Ferulic acid	0.13	0.09
Naringenin	4.94	5.49
Daidzein	0.24	1.03
Querectin	0.51	0.32
Cinnamic acid	0.00	0.04
Apigenin	0.00	3.88
Kaempferol	0.00	0.00
Hesperetin	0.21	0.16

3.5. Proline, total soluble sugar percentage and catalase activity

The highest stressed treatment (2% PEG) showed positive effects on proline, total soluble sugar and catalase activity that were recorded the maximum values (44.02 µg g^{-1}, 21.46% and 26.88 U/g F.W., respectively) in comparison with control as illustrated in Table 4. Using culture medium free of Si (K_2SiO_3 or SiO_2-NPs) individually or under drought condition (2% PEG) caused the highest

proline content and catalase activity. While, adding 2gl^{-1} of K_2SiO_3 enhanced the total soluble sugar to the highest value that also led to the same result by interacting with the highest level of PEG (2%).

Similarly, El-Sayed *et al.* (2022) [44] recorded that drought enhanced proline and total carbohydrate levels in plant. Proline content was increased by using PEG in culture media, whereas adding Si in combined with PEG led to a decrease in proline content compared to other treatments [45]. This could be attributable to an increase in protein degradation and/or the conversion of some amino acids, such as ornithin, arginine, and glutamic acid, to proline. Torabi *et al.* (2015) [46] revealed that, CAT activity was lowered when borage plants treated with Si. Otherwise, under various abiotic stress conditions, the application of Si enhanced the osmoprotective level and the antioxidative activity of many enzymes [47]. Drought enhanced the proline contents, soluble sugar and catalase activities [48].

Table 5. Effect of potassium silicate (K_2SiO_3) and silica nanoparticles (SiO_2-NPs) on prolin, total soluble sugar and catalase activity of *in vitro Antigonon leptopus* plant grown under drought conditions

Treatment A	Treatment B	Proline µg/g	Total sugar %	Catalase U/g F.W.
0% PEG	Control	28.40 J	11.41 J	19.16I
	1gl^{-1} K_2SiO_3	29.30 IJ	13.40 H	21.93G
	2gl^{-1} K_2SiO_3	28.12J	13.75H	20.69H
	2.5mgl^{-1} SiO_2-NPs	31.40H	12.32I	22.69G
	5mgl^{-1} SiO_2-NPs	29.81	13.13H	22.00G
1% PEG	Control	43.32C	15.46G	28.43B
	1gl^{-1} K_2SiO_3	39.09F	17.10F	24.82EF
	2gl^{-1} K_2SiO_3	36.01G	18.45E	22.21G
	2.5mgl^{-1} SiO_2-NPs	40.96E	16.71F	25.84 CDE
	5mgl^{-1} SiO_2-NPs	35.14 G	16.60F	25.25 DE
2% PEG	Control	50.67A	19.75D	30.02A
	1gl^{-1} K_2SiO_3	41.87DE	22.65B	26.46CD
	2gl^{-1} K_2SiO_3	38.30F	24.06A	24.00F
	2.5mgl^{-1} SiO_2-NPs	46.31B	19.89D	27.05C
	5mgl^{-1} SiO_2-NPs	42.96CD	20.94C	26.88C
Mean	0%PEG	29.41C	12.80C	21.29C
	1%PEG	38.91B	16.86B	25.31B
	2%PEG	44.02A	21.46A	26.88A
Mean	Control	40.80A	15.54E	25.87A
	1gl^{-1} K_2SiO_3	36.75C	17.72B	24.40C
	2gl^{-1} K_2SiO_3	34.15E	18.75A	22.30D
	2.5mgl^{-1} SiO_2-NPs	39.56B	16.31D	25.29B
	5mgl^{-1} SiO_2-NPs	35.97D	16.89C	24.71BC

Control: MS+2ip at 0.2mgl^{-1} + IBA at 0.1mgl^{-1}. [Means within a column having the same letters are not significantly different according to Duncan's Multiple Range Test (DMRT) at 5% level]

4. Conclusion

Applying a source of silica (K_2SiO_3 and SiO_2-NPs) can decrease the adverse effects of drought stress under *in vitro* culture resulting from using PEG, thus improving the growth of *Antigonon leptopus.*

Conflicts of interest

There are no conflicts to declare.

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