INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
21–0522/2021/26–4–469–478
DOI: 10.17957/IJAB/15.1858
http://www.fspublishers.org

Full Length Article

Zinc and Silicon Nanomolecules Application Enhances Tolerance to PEG-Induced Drought Stress in Strawberry Cultured *In Vitro*

Isam M Abu Zeid[1†], Fouad H Mohamed[2†] and Ehab MR Metwali[3†*]
[1]*Department of Biological Sciences, Faculty of Science, King Abdulaziz University, P.O. Box 139109, Jeddah 21323, Saudi Arabia*
[2]*Horticulture Department, Faculty of Agriculture, Suez Canal University, 41522 Isamilia, Egypt*
[3]*Genetic Branch, Botany Department, Faculty of Agriculture, Suez Canal University, 41522 Ismailia, Egypt*
[*]For correspondence: ehabmetwali@hotmail.com; ehab_ghareb@agr.suez.edu.eg
†Contributed equally to this work and are co-first authors
Recieved 29 April 2021; Accepted 12 July 2021; Published 28 September 2021

Abstract

Strawberry is highly sensitive to drought, which could limit its cultivation in regions with limited water resources. With unique characteristics, nanoparticles (NPs) could alleviate the negative impacts of abiotic stresses, particularly drought under well-identified environment. The *in vitro* responses of two strawberry cultivars: Sweet Charlie (SW) and Camarosa (CAM) to Zn and Si NPs under polyethylene glycol (PEG)-induced water stress was investigated. Explants were cultured on MS medium amended with three concentrations each of PEG (0, 20 and 40 g L^{-1}) and ZnO NPs (0, 15 and 30 mg L^{-1}) in Exp. 1, or SiO_2 NPs (0, 50 and 100 mg L^{-1}) in Exp. 2. In both experiments, results indicated significant decline in shoot fresh weight, proliferation rate, RWC and chlorophyll content with the increase in PEG level in the medium, up to 40 g L^{-1}. These declines were cultivar-dependent, and SW exhibited better growth performance under drought stress than CAM. Application of ZnONPs at 15 mg L^{-1} or SiO_2NP at 50 mg L^{-1} under drought condition, significantly enhanced the *in vitro* growth and RWC%, and the response of cv. SW to NPs application was higher than CAM. The antioxidant enzymes (CAT and POD) and proline content were highest in the shoots, under the highest levels of Zn or Si NPs. The increase in CAT and proline with ZnONPs application was more in cv SW than CAM. Nano Zn and Si ameliorated drought stress in strawberry through the increase in RWC, antioxidant system and proline accumulation.

Keywords*: Fragaria* x *ananassa* Duch; Proliferation; PEG; Nano ZnO and SiO_2; Catalse; Peroxidase
Abbreviations; BA - Benzyl Adenin; CRD - Complete randomized design; MS - Murashige and Skoog medium; NPs - Nanoparticles; NSC - Number of shoots per proliferated cluster; SFW - Shoot fresh weight; RWC - Relative water vontent; Chl – Chlorophyll; SPSS - Statistical Package for the social sciences; SW - cv.Sweet Cahrlie; CAM - cv.Camarosa; cv – Cultivar; CAT - Catalase; POD - Peroxidase

Introduction

Strawberry (*Fragaria* × *ananassa* Duch) is becoming a popular small fruit in the gulf states and widely grown in the Midditerranian area. Due to its shallow root system, high fruit water content and wide leaf area, the plant is extremely sensitive to drought (Hancock 1999), which may limit its cultivation in region with water shortage. Drought in arid regions, such as Saudi Arabia, adversely affects plant growth, development, productivity and limits culture and germplasm exchange for most crops, including strawberry (Sardhara and Mehta 2018; Zhang *et al*. 2018; Saijo and Po-iian Loo 2020). Cultivar-dependent variations in abiotic stress toleranc have been reported in strawberry plants under *in vivo* (Klamkowski and Treder 2008; Tohmaa and Esitkena 2011) or *in vitro* conditions (Hussein *et al*. 2017). Therefore, the choice of relatively tolerant genotype would be of great impact in future breeding and extention efforts to help expand strawberry cultivation under conditions of water stress.

Studies on plant responses to drought stress in the field may be associated with non-uniform moisture availability and temperature fluctuations during the growing season. In addition, the described method requires a lot of planting space, time, material resources and equipment (Arvin and Donnelly 2010). In such studies, *in vitro* screening for abiotic stress tolerance would be beneficial to reduce the effect of changing external environments, screening of large number of genotypes under limited space and well-identified environment (Shatnawi *et al*. 2004; Bednarek and Orłowska 2020).

To cite this paper: Zeid IMA, FH Mohamed, EMR Metwali (2021). Zinc and silicon nanomolecules application enhances tolerance to peg-induced drought stress in strawberry cultured *in vitro*. *Intl J Agric Biol* 26:469–478

The utilization of nanomaterials is expected to help overcome many of the problems related to plant propagation (Helaly *et al*. 2014) and production (Nair *et al*. 2010; Zahedi *et al*. 2020b). Two of these nanoparticles (NPs) signaling molecules, SiO_2 NPs and ZnONPs have been proved to play an active role, in inducing many physiological and biochemical changes within the cell, and allowing them to overcome stresses, including drought or salt stress (Marslin *et al*. 2017; Sun *et al.* 2020). Exogenous application of nanoelements has shown positive effects on growth and productivity, as well as enahnced tolerance to drought stress in several crops, including strawberry (Mozafari *et al.* 2018; Zahedi *et al*. 2020a); tomato (Faizan *et al.* 2018); potato (Mahmoud *et al.* 2020); pepper (García-López *et al.* 2019), among other crops. However, depending on the concentration and type of NPs, growing conditions, and species, previous research on the influence of NPs on plant growth revealed both positive and negative effects (Gruyer *et al.* 2013).

Under environmental stress, the plants are subjected to significant alterations in enzyme activities, and consequently their metabolism, causing increased production of reactive oxygen species (ROS) (Hasegawa *et al*. 2000; Parida and Das 2005), ultimately, this slows plant growth and increases damage to various parts of the plant. To overcome the abiotic stress and minimize the effects of oxidative stress, plants have evolved a variety of strategies for dealing with stressful conditions *via* synthesis of ROS scavenging. In this respect, the stimulation of the plant antioxidant capacity is one of the most important mechanisms of protection against the harmful impact of oxygen radicles (Sherwin and Farrant 1998). Peroxidase (POD) and catalase (CAT) are antioxidant enzymes that widely known by their high antioxidative effects (Apel and Hirt 2004; Kruk *et al*. 2005; Ikeda *et al*. 2011). In addition, some studies have suggested the amino acid proline (Bano *et al.* 2013) or chlorophyll accumulation (Dehghanipoodeh *et al*. 2018) to serve as indicator to abiotic stresses. However, further reaserch is required to monitor the relative changes in these biochemicals under the influnce of NPs treatments.

The present studies assessed the possible alleviation of drought stress by applying Zn and Si nanomolecules to strawberry (*Fragaria* × *ananassa* Duch) cultivras grown under PEG-induced water stress *in vitro*, and determine their relative tolerance to drought stress. Alterations on some physiological and biochemical markers associated with the application of NPs under drought stress was also investigated.

Materials and Methods

Plant materials

In vitro culture system was carried out at the Genomic and Biotechnology Laboratory, Department of Biological Sciences, King Abdulaziz University, KSA in collaboration with Plant Tissue Culture Unit, Horticulture Department, Faculty of Agriculture, Suez Canal University, Egypt during the period from March 2018 to May 2019. The two strawberry (*Fragaria* x *ananassa* Duch.) cultivars *i.e.*, Sweet Charlie (SW) and Camarosa (CAM) were examined in the following two experiments.

Preparation of ZnO-NPs and SiO2-NPs suspension

The two nanomaterials, ZnO and SiO_2 (Sigma–Aldrich Company, California, USA and Nanotechnology Unit, Beni-Sueif University, Egypt) were utilized in the current study. Suspensions of ZnONPs and SiO_2NPs were freshly prepared with distilled water and dispersed with a sonicator for 30 min at two concentrations of 15 and 30 mg L^{-1} and 50 and 100 mg L^{-1}, respectively. Before being applied to the culture media, the nanoparticle suspensions were centrifuged (Helaly *et al.* 2014; Gowayed *et al*. 2017).

In vitro propagation

Sterilized runner meristem tip explants of the two strawberry cultivars were cultured *in vitro*, under aseptic conditions on shoot initiation medium composed of MS salts (Murashige and Skoog 1962), 3% sucrose, 100 mg L^{-1} myo-inositol, and 1 mg L^{-1} thiamin-HCl. After adjusting the pH of the medium to 5.7, it was solidified with 0.7 percent agar and autoclaved at 121ºC for 20 min at 15 psi. Cultures were maintained in a growth chamber for 4 weeks at 22 ± 2°C and 16 h photoperiod with an irradiance of 45 μmol m^{-2} s^{-1} provided by white fluorescent lamps. To obtain enough stock plantlets for future experiments, meristem-derived plantlets were sub-cultured onto shoot multiplication MS medium supplemented with 0.3 mg L^{-1} BA, 3% sucrose and incubated on the shelves of a growth room at the same conditions as above.

Response of strawberry to *in vitro* PEG-induced water stress and nanomolecules

Single plantlets excised from the proliferated shoot clusters of the two strawberry cultivars were subcultured onto glass jars (3 explants per jar) containing MS medium (30 mL per jar) supplemented with 0.3 mg L^{-1} BA. Water stress was imposed by the addtion into the medium of 3 concentrations of PEG 6000 (0, 20 and 40 g L^{-1}), while stress alleviation was examined by supplemting the same medium with ZnONPs at 0, 15 and 30 mg L^{-1} (Exp. 1), or SiO_2NPs at 0, 50 and 100 mg L^{-1} (Exp. 2). Media preparation and culture conditions were similar to the above. Each experiment was 2*3*3 factorial in a complete randomized design (CRD) and five replicates. In both experiments, fully proliferated shoot clusters were obtained at six weeks from culture initiation. Data were taken on shoot cluster fresh weight (SFW) and number of shoots per cluster (NSC) using five randomly selected replicates (clusters) per treatment.

Relative water content, chlorophyll and proline determination

4*4 mm leaf disks were used to assess relative water content (RWC) and after calculating the fresh weight (FW), they were submerged overnight in distilled water, blotted dry on a paper towel, and the turgid weight (TW) was calculated. After drying for 48 h at 70°C, the sample dry weight (DW) was determined and the RWC was calculated using the formula:

$$\% \text{ RWC} = (\text{FW-DW})/(\text{TW-DW}) \times 100.$$

Chlorophyll was determined according to Lichtenthaler (1987). Leaves from the proliferated shoots (0.5 g) were homoginized in 80% acetone and the extracts were centrifuged at 3000 x g. Absorbance was recorded at 644 and 662 nm for chl *a* and chl *b* assay, respectively, using spectrophotometer model Unico UV/VIS 2100.

For proline determination, plant material (0.1 g) was homogenized in 2 mL of 3% aqueous sulphosalicylic acid before being filtered through Whatman No. 2 filter paper. 2 mL of filterate was mixed with 2 mL of glacial acetic acid and 2 mL of acid ninhydrin before being heated in a boiling water bath for 1 h. Place the tub in an ice bath to stop the reaction, then add 4 mL of toluene to the reaction mixture and stir for 20 sec. The red color intensity was measured at 520 nm after the toluene layer was separated (Sadasivam and Manickam 1991).

Quantitative determination of antioxidant enzymes

Plant samples (0.1–0.4 g *in vitro* shoots) were stored at -20°C before being processed according to Ni *et al*. (2001). Hammerschmidt *et al*. (1982) method was used to assess peroxidase (POD) activity. 1.5 mL pyrogallol (0.05 *M*) and 100 μL enzyme extract were applied to a spectrophotometer sample cuvette. At 420 nm, the reading was set to zero. The reaction was started by adding 100 μL of hydrogen peroxide (1%) to the sample cuvette. The activity of catalase (CAT) was determined using biodiagnostic kit No. CA 2517, which is based on Aebi (1984). spectrophotometric method. Catalase reacts with a known amount of hydrogen peroxide, and catalase inhibitor stops the reaction after 1 min. In the presence of peroxidase, the remaining H_2O_2 reacts with 3,5-dichloro-2-hydroxybenzene sulfonic acid and 4-aminphoenazone to form a chromophore with a color intensity inversely proportional to the amount of catalase in the sample to form a chromophore with a color intensity inversely proportional to the amount of catalase in the sample. The absorbance was assessed at 510 nm.

Statistical analysis

The experiments were 2 x 3 x 3 factorial in CRD design. In each experiment, data were subjected to the analysis of variance (ANOVA) with the aid of SPSS 14 for windows statistical package (IBM Corp., New York, USA). Means were compared with Duncan's multiple range test at $P \geq 0.05$ (Snedecor and Cochran 1989).

Results

Experiment No. 1

Effects on the *in vitro* growth and shoot proliferation

Results of ANOVA for *in vitro* shoot cluster fresh weight (SFW) and proliferation potential, expressed as number of shoots/cluster (NSC), of the two strawberry cultivars: Sweet Charlie (SW) and Camarosa (CAM) in response to ZnONPs treatments under PEG-induced drought stress are shown in (Table 1). Results indicated that SFW was significantly affected by cultivar, PEG and ZnONPs treatments. The cv SW had 16% higher SFW and 72% more NSC than cv CAM. Drought stress induced by PEG at 20 or 40 g L^{-1} significantly decreased SFW by 30 and 41%, respectively, compared to the control. Application of ZnONPs, especially at 30 mg L^{-1}, slightly reduced SFW and NSC when tested over cultivars and PEG levels. All interactions had no significant effect on SFW (Table 1). However, it could be estimated (Fig. 1A) that, under sever water stress (40 g L^{-1} PEG), SFW was decreased in both cultivars, in varing degree (41% in cv SW and 53% in CAM). With the application of ZnONPs at 15 mg L^{-1} under sever water stress, SFW was enhanced in both cultivars (Fig. 1A). Under moderate water stress (20 g L^{-1} PEG), the same ZnONPs treatment improved SFW in cv CAM by 17.4%. The interaction of CV * PEG * ZnONPs significantly affected NSC, and the application of ZnONPs at 15 mg L^{-1} under severe drought, had resulted in increased NSC by 18.8% (compared to 0 ZnONPs) in cv SW, while had no effect on CAM, indicating different magnitute of cultivar response to PEG * ZnONPs treatments (Fig. 1B).

Effects on RWC, Chl, antioxidant enzymes and proline

The RWC was significantly higher in cv SW than CAM (Fig. 1C). Drought stress (40 g L^{-1} PEG) significantly decreased RWC, and this decline was more in cv CAM (13.2%) than SW (6.47%), as indicated by the significant CV * PEG interaction (Table 2 and Fig. 2A). In contrast, the application of ZnONPs at 15 mg L^{-1} increased RWC and the increase was more in cv SW than CAM (Table 2 and Fig. 3A).

ANOVA revealed that cultivar and PEG-induced water stress treatments had significant main effects on chlorophyll *a+b* (Chl) content in strawberry shoots (Table 2). The cv SW had more Chl than CAM and the increasing level of PEG, up to 40 g L^{-1} had resulted in signgificant decrease in Chl by 61.6% (Tables 2, 3 and Fig. 1A). This decline was found to be more in cv CAM than SW (Fig. 2B). Under moderate and severe water stress (PEG at 20 and 40 g L^{-1}, respectively), ZnONPs at 15 mg L^{-1} caused stability or

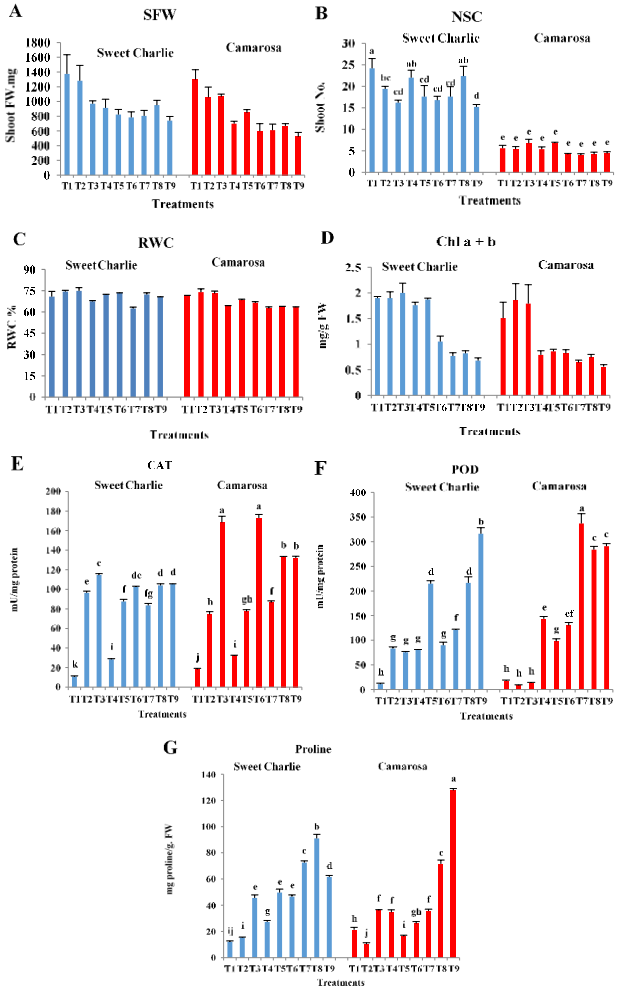

Fig. 1: Three-way interaction effects of CV*PEG*ZnONPs on **A**) SFW; **B**) NSC; **C**) RWC; **D**) Chl; **E**) CAT; **F**) POD and **G**) proline in two strawberry cultivars.

T1 = Control, T2 = 15 mg L^{-1} ZnONp, T3 = 30 mg L^{-1} ZnONP, T4 = 20 g L^{-1} PEG, T5 = 20 g L^{-1} PEG + 15 mg L^{-1} ZnONP, T6 = 20 g L^{-1} PEG + 30 mg L^{-1} ZnONP, T7 = 40 g L^{-1} PEG, T8 = 40 g L^{-1} PEG + 15 mg L^{-1} ZnONP, T9 = 40 g L^{-1} PEG + 30 mg L^{-1} ZnONP

slight increase in Chl content (Fig. 1D).

The activities of CAT and POD as well as proline contents in strawberry were significantly affected by cultivar, PEG and ZnONPs treatments (Table 2 and Fig. 1E, F and G). Shoots of cv CAM had more activity of CAT and POD than SW. However, proline content was significantly higher in cv SW. Under severe water stress, the activities of CAT, POD and proline contents were increased ($P \leq 0.001$, Table 2) by 9.7, 86.5 and 38.8%, respectively. Application of ZnONPs at 15 and 30 mg L^{-1} significantly increased CAT by 54.7 and 71% and POD by 1.2 and 22.4%, respectively (Table 2 and Fig. 1E and F).

The CV * PEG interaction significantly affected CAT and POD activities (Table 2 and Fig. 2C and D) and shoots of cv CAM recorded higher increase in CAT and POD under severe water stress than SW. Similar trend was observed for proline content (Fig. 2E). Meanwhile, the two-

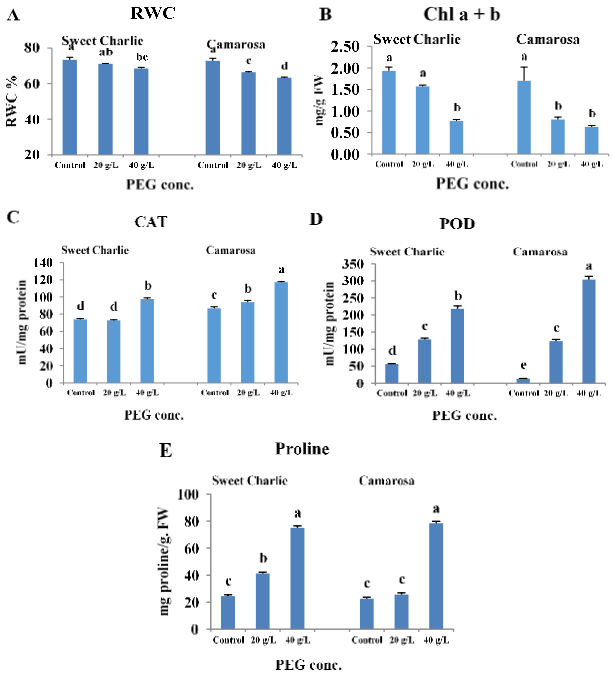

Fig. 2: Effect of CVxPEG on **A**) RWC, **B**) Chl , **C**) CAT, **D**) POD and **E**) proline in two strawberry cultivars.

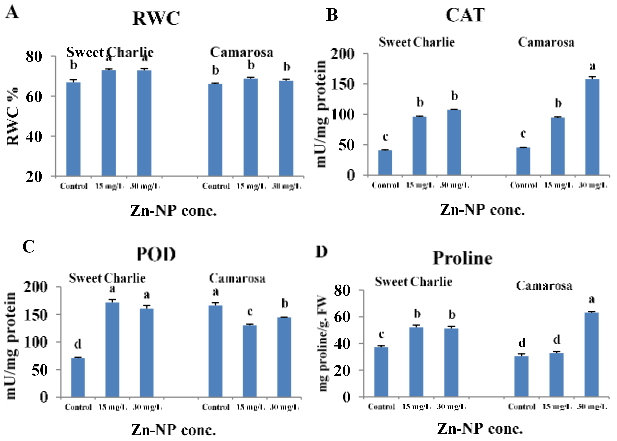

Fig. 3: Effect of CV*ZnONPs on **A**) RWC, **B**) CAT, **C**) POD, **D**) and proline in two strawberry cultivars.

way ANOVA indicated significant CV * ZnONPs interaction effect on antioxidant enzymes and proline (Table 2). In this regard, the application of ZnONPs at 30 mg L^{-1} resulted in more increase in CAT activity (Fig. 3B) and proline content (Fig. 3D) in cv CAM than SW, while the reverse was true for POD activity (Fig. 3C).

A significant PEG * ZnONPs effect was detected on the activities of CAT, POD and proline contents (Table 2; Fig.3B, 4). The highest CAT was found in shoots exposed to ZnONPs at 15 mg L^{-1} under non- and moderate water stress conditions (Fig. 4A), while POD (Fig. 4B) and proline (Fig. 4C) were the highest in shoots exposed to ZnONPs at

Table 1: Summary of analysis of variance for *in vitro* shoot fresh weight and shoot number in two strawberry cultivars under different treatments of PEG and ZnONPs

SOV	df	F value	
		SFW	NSC
Cultivars (CV)	1	7.56^{**}	515.79^{***}
PEG	2	30.97^{***}	2.39^{ns}
Zn	2	4.52^{*}	6.46^{**}
CV * PEG	2	0.85^{ns}	0.19^{ns}
CV * Zn	2	0.14^{ns}	6.54^{**}
PEG * Zn	4	1.02^{ns}	2.10^{ns}
CV*PEG*Zn	4	0.86^{ns}	3.30^{*}
Error	72		
Total	89		
CV%		27.55%	23.85%

Table 2: Summary of analysis of variance for RWC, Chl, CAT, POD and proline in two strawberry cultivars under different treatments of PEG and ZnONPs

SOV	df	F value				
		RWC	Chl	CAT	POD	Proline
Cultivars (CV)	1	29.20^{***}	22.82^{***}	281.19^{***}	14.07^{***}	34.76^{***}
PEG	2	43.45^{***}	76.69^{***}	255.51^{***}	1493.76^{***}	1664.57^{***}
Zn	2	18.62^{***}	2.30^{ns}	2354.08^{***}	42.84^{***}	289.87^{***}
CV * PEG	2	5.34^{**}	6.83^{**}	5.63^{**}	126.87^{***}	49.06^{***}
CV * Zn	2	4.52^{*}	1.54^{ns}	233.30^{***}	152.95^{***}	127.83^{***}
PEG * Zn	4	0.51^{ns}	1.64^{ns}	245.34^{***}	26.35^{***}	84.43^{***}
CV*EPG*Zn	4	1.38^{ns}	1.54^{ns}	55.68^{***}	50.63^{***}	244.86***
Error	36					
Total	53					
CV%		3.37%	21.99%	4.35%	8.89%	6.60%

Each column shows significant differences at $P \leq 0.05$ (*), $P \leq 0.01$(**), and $P \leq 0.001$ (***) between three-factor factorial (i) cutivars, (ii) PEG, (iii) Zn by Duncan's multiple range test (DMRT); ns – non-significant difference.

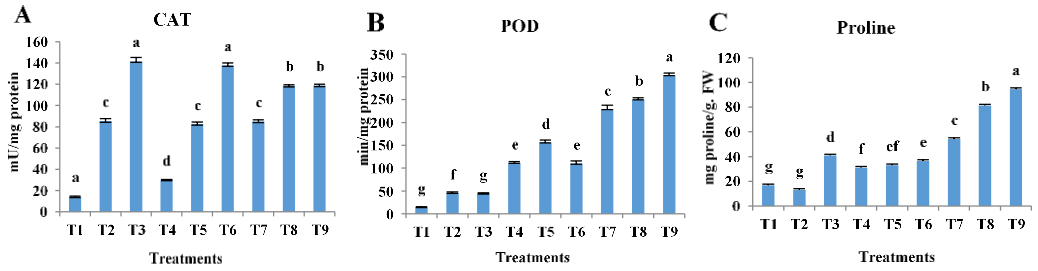

Fig. 4: Effects of PEG x* ZnONPs on **A**) CAT, **B**) POD and **C**) proline content in two strawberry cultivars.
T1 = Control, T2 = 15 mg L^{-1} ZnONp, T3 = 30 mg L^{-1} ZnONP, T4 = 20 g L^{-1} PEG, T5 = 20 g L^{-1} PEG + 15 mg L^{-1} ZnONP, T6 = 20 g L^{-1} PEG + 30 mg L^{-1} ZnONP, T7 = 40 g L^{-1} PEG, T8 = 40 g L^{-1} PEG + 15 mg L^{-1} ZnONP, T9 = 40 g L^{-1} PEG + 30 mg L^{-1} ZnONP

30 mg L^{-1} under svere water stress. ANOVA also indicated significant CV * PEG * ZnONPs interaction effects on CAT and POD activities and proline content (Table 2). The highest CAT was recorded in cv CAM under non-stress or moderate stress conditions combined with ZnONPs at 30 mg L^{-1} (Fig. 1E). Application of ZnONPs at 15 or 30 mg L^{-1} under water stress, resulted in more increase in CAT than the observed increse induced by PEG alone. This was also true for POD activity (Fig. 1F) and proline content (Fig. 1G).

Experiment 2

Effects on the *in vitro* growth and shoot proliferation

The results indicated significant increase in growth performance of cv SW over CAM in terms of SFW (16.2%) and NSC (72%). PEG-induced water stress at 20 and 40 g L^{-1} resulted in significant decrease in SFW by 35.5 and 42%, respectively over the control. Application of SiO_2NPs at 50 mg L^{-1} significantly increased SFW (15.7%) and NSC (23%). A significant CV * SiO_2NPs interaction effect was detected on NSC, and the increase in NCS at 50 mg L^{-1} was more in cv SW than CAM (Table 3; Fig. 7A). The interaction of CV * PEG * SiO_2NPs did not affect SFW and NSC (Fig. 5). However, SiO_2NPs improved SFW and NSC (Fig. 9b) in both cultivars, under moderate water stress.

Effects on RWC, Chl, antioxidant enzymes and proline

Results indicated significant decline in RWC % under high level of PEG-induced drought. The application of SiO_2NPs

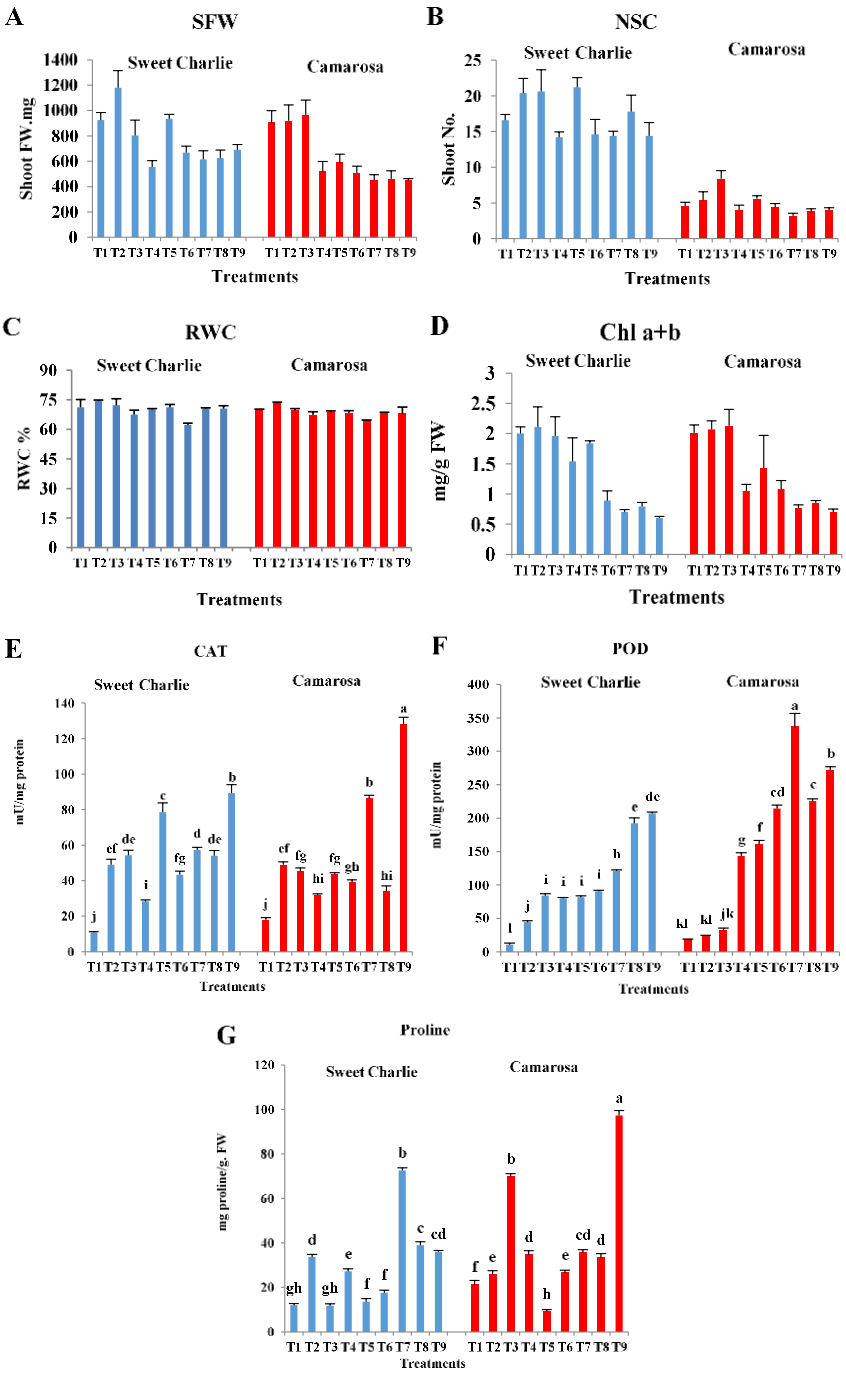

Fig. 5: Three-way interaction effect of CV*PEGxSi-NPs on **A**) SFW; **B**) NSC; **C**) RWC; **D**) Chl; **E**) CAT; **F**) POD and **G**) proline in two strawberry cultivars.

T1 = Control, T2 = 50 mg L^{-1} SiO_2NPs, T3 = 100 mg L^{-1} SiO_2NPs, T4 = 20 g L^{-1} PEG, T5 = 20 g L^{-1} PEG + 50 mg L^{-1} SiO_2NPs, T6 = 20 g L^{-1} PEG + 100 mg L^{-1} SiO_2NPs, T7 = 40 g L^{-1} PEG, T8 = 40 g L^{-1} PEG + 50 mg L^{-1} SiO_2NPs, T9 = 40 g L^{-1} PEG + 100 mg L^{-1} SiO_2NPs

significantly increased RWC%, and the increase was more in cv SW than CAM (Table 4).

Accumulation of Chl *a+b* in strawberry shoots was significantly decreased under PEG-induced water stress at 20 and 40 g L^{-1} by 36 and 64%, respectively. On the other hand, the application of SiO_2NPs at 50 mg L^{-1} significantly increased Chl content by 11.3%. Under moderate water stress, SiO_2NPs at 50 mg L^{-1} enhanced Chl in shoots of both strawberry cultivars (Fig. 5D).

Drought stress, induced by PEG at 40 g L^{-1}, significantly ($P \leq 0.001$) increased the activities of CAT, POD and proline content in shoots of strawberry. With the

Table 3: Summary of analysis of variance for *in vitro* shoot fresh weight and shoot number in two strawberry cultivars under different treatments of PEG and SiO_2NPs

SOV	df	F value	
		SFW	NSC
Cultivars (CV)	1	13.75^{***}	356.26^{***}
PEG	2	43.56^{***}	7.60^{**}
Si	2	4.27^{**}	6.46^{**}
CV * PEG	2	1.70^{ns}	0.34^{ns}
CV * Si	2	2.62^{ns}	3.85^{*}
PEG * Si	4	1.42^{ns}	2.31^{ns}
CV*PEG*Si	4	1.54^{ns}	0.18^{ns}
Error	72		
Total	89		
CV%		24.81%	28.18%

increasing level of SiO_2NPs, up to 100 mg L^{-1}, the activities of both antioxidant enzymes CAT and POD, as well as proline content were also increased by 35.1, 21, and 21.5%, respectively (Table 4). The two-way ANOVA indicated significant ($P \leq 0.001$) CV *PEG effects on the activities of CAT, POD and proline content (Table 4, Fig. 6). In this regard, the increase over control treatment in CAT (Fig. 6A) and POD (Fig. 6B) under severe water stress was more in cv CAM than SW, in contrast to proline content which was higher in cv SW than CAM (Fig. 6C). The two strawberry cultivars significantly ($P \leq 0.001$) recorded different CAT and POD activities in response to SiO_2NPs treatment (Fig. 7B and C). In contrast to cv CAM, the cv SW had shoots with lower proline content with the increase in SiO_2NPs level in the medium (Fig. 7D). The interaction of PEG * SiO_2NPs significantly ($P \leq 0.001$) affected CAT, POD and proline, which were the highest under severe water stress and 100 mg L^{-1} SiO_2NPs treatment (Fig. 8A, B and C). A significant CV * PEG * SiO_2NP interaction effect on antioxidant enzyems and proline (Table 4) indicated differences in the magnitute of increase in CAT and POD activities and proline content between the two examined strawberry cultivars under the influence of drought stress and SiO_2NPs levels (Fig. 5E, F and G, respectively).

Discussion

In the present study, shoot growth and prolieferation potential, physiological changes and antioxidant enzymes activities in two strawberry cultivars were examined in response to the application of ZnONPs or SiO_2NPs under drought stress. Results revealed that drought imposed by PEG treatment had resulted in significant decline in shoot FW, number of shoots/cluster (NSC) and leaves RWC. Similar findings were reported by Gopal and Iwama (2007) on *in vitro* screening of potato under drought stress. The decline in RWC and subsequantly in shoot FW may be due to restricted water and nutrient absorption as a result of decrease in water potential of the medium supplemented with PEG, and/or cell elongation suppression due to low turger pressure (Jaleel *et al.* 2009). Drought stress imposed by PEG also reduced growth of *in vitro*-grown grapevine, decrease indole acetic acid (IAA) and increase abscisic acid (ABA) levels (Cui *et al.* 2016). This growth promoter/inhibitor imbalance contributed to the obsereved reduction in (NSC) in strawberry under water stress. Drought stress had a negative impact on plant growth parameters including leaf area, plant height, and stem diameter by altering a number of morphological, physiological and metabolic processes (Fard *et al*. 2011). García-Sánchez *et al*. (2002) reported that the reduction in growth under water stress might be due to ion toxicity and imbalance, or change in growth regulators biosynthesis. Hence, any water deficit leads to a growth pause (Aslanpour *et al*. 2019).

Strawberry cultivars examined in the current study showed differential response to water stress condition and cv SW exhibited lower PEG-induced reduction in SFW, NSC, RWC and chlorophyll content, than cv CAM, at the highest level of PEG-induced water stress. In addition, the CAT activity and proline accumulation were more in shoots of cv SW than in CAM. In several reports, plants exposed to water stress showed enhanced proline content (Sultan *et al.* 2012) or antioxidant enzyme activity (Yosefi *et al.* 2020) were identified as tolerant to drought, which may explain the relative tolerance of cv SW to drough stress compared to cv CAM. Similar variation in antioxidant enzyme activities were reported in strawberry cultivars exposed to salt stress (Turhan *et al.* 2008).

Our results also indicated significant enhancement of shoot proliferatin and RWC of strawberry with the application of ZnONPs at 15 mg L^{-1} in cv SW or at 30 mg L^{-1} in cv CAM under PEG-induced drought stress (Fig. 9a). This increased shoot proliferation ability with ZnONPs under water stress could be attributed to Zn's function in improving plant water status (Helaly *et al*. 2014), as well as management of reactive oxygen species and protection of plant cells from oxidative stresses (Sheikh *et al*. 2009). Recently, it was suggested that modifications in the endogenous melatonin synthesis were invoved in the ZnONPs induced drought tolerance in maize (Sun *et al.* 2020). In accordance with present study results, *in vitro* callus induction and plant regeneration were increased in tomato cultivars undere abiotic stress in the presence of ZnONPs in the medium (Alharby *et al.* 2016).

Results also showed that SiO_2NPs treatments significantly incresed shoot FW, NSC, RWC and chlorophyll accumulation under PEG-induced water stress, but with different degrees of influence between strawberry cultivars (Fig. 9b). This beneficial effect could be attributed to silicon treatment, which can raise gibberellic acid levels in cells and has a plant hormone-like property that could aid cell division and elongation (Soundararajan *et al*. 2014). Previous studies have shown that applying Si-NPs to plants under saline stress increased chlorophyll content, stomatal conductance and plant water use quality (Hattori *et al*. 2005; Ahmed *et al*. 2011). In accordance with present study results, application of SiONPs to strawberry plants accumulated high proline under water stress, assoicated with the tolerance mechanism (Dehghanipoodeh *et al*. 2018).

Table 4: Summary of analysis of variance for RWC, Chl, CAT, POD and proline in two strawberry cultivars under different treatments of PEG and SiO_2NPs

SOV	df	F value				
		RWC	Chl	CAT	POD	Proline
Cultivars (CV)	1	2.60^{ns}	0.13^{ns}	0.83^{ns}	432.55^{***}	239.38^{***}
PEG	2	10.30^{***}	53.59^{***}	345.81^{***}	1590.16^{***}	811.32^{***}
Si	2	7.50^{**}	2.58^{*}	169.82^{***}	53.39^{***}	239.76^{***}
CV * PEG	2	0.080^{ns}	0.94^{ns}	43.09^{***}	207.59^{***}	58.71^{***}
CV * Si	2	1.29^{ns}	0.84^{ns}	63.74^{***}	50.28^{***}	635.61^{***}
PEG * Si	4	1.79^{ns}	1.07^{ns}	165.64^{***}	7.50^{***}	131.50^{***}
CV*EPG*Si	4	0.23^{ns}	0.34^{ns}	22.06^{***}	58.27^{***}	174.43^{***}
Error	36					
Total	53					
CV%		4.31%	27.93%	8.65%	7.78%	6.95%

Each column shows significant differences at $P \leq 0.05$ (*), $P \leq 0.01$(**), and $P \leq 0.001$ (***) between three-factor factorial (i) cutivars, (ii) PEG, (iii) Zn by Duncan's multiple range test (DMRT); ns – non-significant difference.

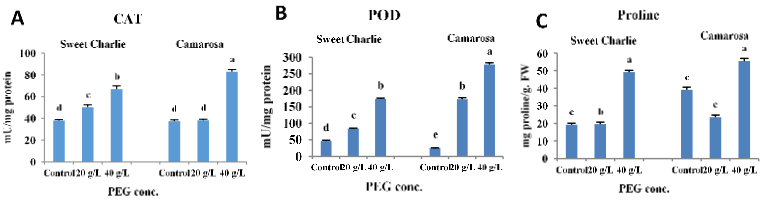

Fig. 6: Effect of CV* PEG on **A**) CAT, **B**) POD and **C**) proline in two strawberry cultivars.

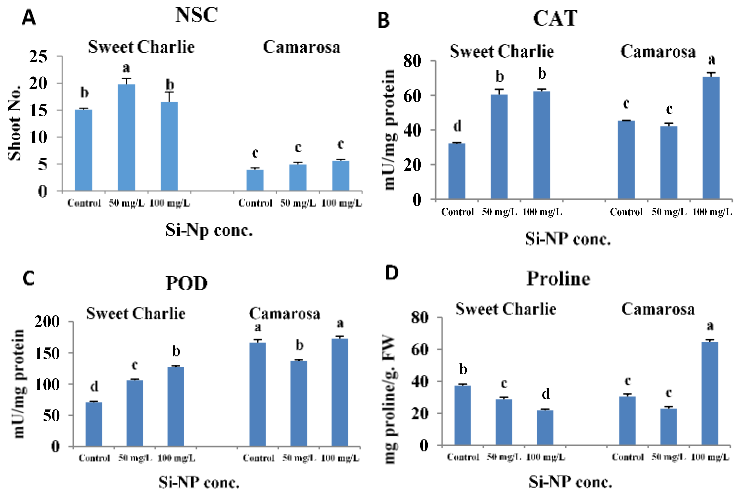

Fig. 7: Effect of CV * SiO_2NPs on **A**) NSC, **B**) CAT, **C**) POD and **D**) proline in two strawberry cultivars

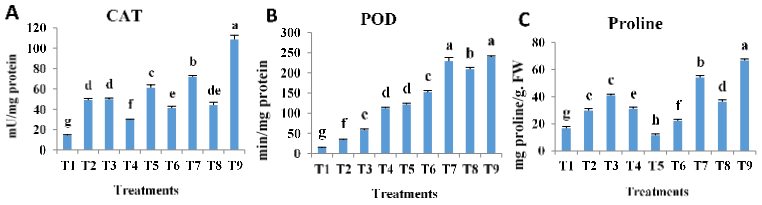

Fig. 8: Effect of PEG * SiO_2NPs on **A**) CAT, **B**) POD and **C**) proline in two strawberry cultivars.
T1 = Control, T2 = 50 mg L^{-1} SiO_2NPs, T3 = 100 mg L^{-1} SiO_2NPs, T4 = 20 g L^{-1} PEG, T5 = 20 g L^{-1} PEG + 50 mg L^{-1} SiO_2NPs, T6 = 20 g L^{-1} PEG + 100 mg L^{-1} SiO_2NPs, T7 = 40 g L^{-1} PEG, T8 = 40 g L^{-1} PEG + 50 mg L^{-1} SiO_2NPs, T9 = 40 g L^{-1} PEG + 100 mg L^{-1} SiO_2NPs

In the present study, results showed significant increase in CAT and POD activity as well as proline contents, especially under severe drought and high levels of NPs. Recent report indicated that plants may protect themselves from drought stress by accumulating compatible solutes such as sugars, amino acids and enzymes for osmotic adjustment (Khodabin *et al.* 2020). According to Ashraf and Foolad (2007) and Gao *et al.* (2020), enzyme

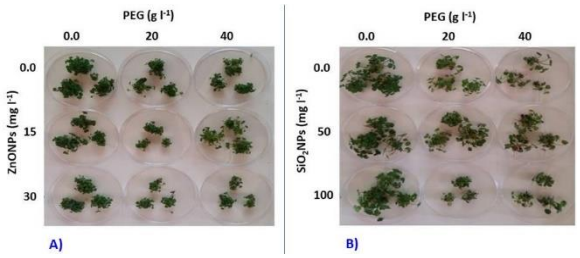

Fig. 9: Infleunce of different concentrations of **A**) ZnONPs and **B**) SiO_2NPs on the *in vitro* growth and shoot proliferation in strawberry cv. Sweet Charlie under PEG-induced drought, 4 weeks from culture initiation

activity will appear to be scavenging of phospholipid hydroperoxides, thereby protecting cell membranes from peroxidative damage under abiotic stress and improving the ability of plant tissues to scavenge O_2 radicals. Our results are also in agreement with Bano *et al.* (2013), who recognized proline as one of the most common amino acid accumulating as a result of disturbances in osmotic balance, and it can serve as an indicator of abiotic stress. Other studies have linked the accumulation of proline to drought stress in strawberry (Radhi and Abudl-Hasan 2020), which could play a protective role against the osmotic potential generated by water stress (Farooq *et al*. 2012).

The variation between strawberry cultivars in their response to different treatments, whether drought or other NPs was reported in earlier studies under field conditions (Klamkowski and Treder 2008), which may explain the inconsistent findings of our study. Strawberry cv Sweet Charlie is largely preferred by strawberry growers in our region for its high yield and early fruiting habit. The current study has provided evidence of the relative tolerance of strawberry to drought stress, especially under the influence of ZnONPs and SiO_2NPs at early stage of *in vitro* growth, which may help expanding strawberry propagation and/or cultivation using micro-propagated stock plants, better adapted to drought conditions.

Conclusion

Negative consequences of drought stress on the *in vitro* growth and proliferation ability in strawberry were cultivar-dependent and could be alleviated by application of ZnONPs or SiO_2NPs. This enhanced tolerance was accompanied by increases in RWC and activities of antioxidant enzymes: catalase and peroxidase and increased accumulation of proline content in shoots of drought-affected plants. Further studies are needed to explain the relashionship between the *in vitro* and *ex vitro* respones of strawberry to nanomaterials under abiotic stresses.

Acknowledgements

This project was funded by the Deanship of Scientific Research (DSR) at King Abdulaziz University, Jeddah, under grant no. (G: 355-130-1440). The authors, therefore, acknowledge DSR for technical and financial support.

Author Contributions

Isam Abu Zeid conceived the research, designed the figures and tables and participated in the discussion results, reviewed several draft of the manuscript, read and approved the manuscript and Fouad Mohamed and Ehab Metwali performed the experiment, analyzed data, conducted statistical analysis and wrote the manuscript.

Conflict of Interest

The author declear that they have no conflict of interest.

Data Availability

The author affirm that data will be available on a fair request to the corresponding author

Ethics Approval

Not applicable to this paper

Referances

Aebi H (1984). Catalase *in vitro*. *Meth Enzymol* 105:121–126

Ahmed M, FU Hassen, U Qadeer, MA Aslam (2011). Silicon application and drought tolerance mechanism of sorghum. *Afr J Agric Res* 6:594–607

Alharby HF, EMR Metwali, MP Fuller, AY Aldhebiani (2016). Impact of application of zinc oxide nanoparticles on callus induction, plant regeneration, element content and antioxidant enzyme activity in tomato (*Solanum lycopersicum* Mill.) under salt stress. *Arch Biol Sci* 68:723–735

Apel K, H Hirt (2004). Reactive oxygen species: Metabolism, oxidative stress, and signal transduction. *Annu Rev Plant Biol* 55:373–399

Arvin MJ, DJ Donnelly (2010). Screening potato cultivars and wild species to abiotic stresses using an electrolyte leakage bioassay. *J Agric Sci Technol* 10:33–42

Ashraf M, R Foolad (2007). Roles of glycine betaine and proline in improving plant abiotic stress resistance. *Environ Exp Bot* 59:206–216

Aslanpour M, HD Baneh, A Tehranifar, M Shoor (2019). Effect of water stress on growth traits of roots and shoots (fresh and dry weights, and amount of water) of the white seedless grape. *Intl Tran J Eng Manage Appl Sci Technol* 10:169–181

Bano Q, N lyas, A Ano, N Zafar, A Akram, F Hassan (2013). Effect of Azospirillum inoculation on maize (*Zea mays* L.) under drought stress. *Pak J Bot* 13:13–20

Bednarek PT, R Orłowska (2020). Plant tissue culture environment as a switch-key of (epi)genetic changes. *Plant Cell Tiss Org* 140:245–257

Cui ZH, WL Bi, XY Hao, Y Xu, PM Li, MA Walker, QC Wang (2016). Responses of *in vitro*-grown plantlets (*Vitis vinifera*) to grapevine leafroll-associated virus-3 and PEG-induced drought stress. *Front Physiol* 7; Article 203

Dehghanipoodeh S, C Ghobadi, B Baninasab, M Gheysari, S Bidabadi (2018). Effect of silicon on growth and development of strawberry under water deficit conditions. *Hortic Plant J* 4:226–232

Faizan M, A Faraz, M Yusuf, S Khan, S Hayat (2018). Zinc oxide nanoparticle-mediated changes in photosynthetic efficiency and antioxidant system of tomato plants. *Photosynthesis* 56:678–686

Fard FD, D Habibi, MD Fard (2011). Effect of plant growth promoting rhizobacteria and foliar application of amino acids and salicylic acid on biochemical biomarkers activity of wheat under drought stress. *In: Proceedings of the 2nd International Conference on Chemical Engineering and Applications IPCBEE 2011*, Vol. 23. IACSIT Press, Singapore

Farooq M, M Hussain, A Wahid, KHM Siddique (2012). Drought stress in plants: An overview. *In: Plant Responses to Drought Stress*, pp:1–33. Aroca R (Ed). Springer, New York, USA

Gao S, Y Wang, S Yu, Y Huang, H Liu, W Chen, X He (2020). Effects of drought stress on growth, physiology and secondary metabolites of two *Adonis* species in Northeast China. *Sci Hortic* 259:108795

García-López JI, G Niño-Medina, E Olivares-Sáenz, RH Lira-Saldivar, ED Barriga-Castro, R Vázquez-Alvarado, PA Rodríguez-Salinas, F Zavala-García (2019). Foliar application of zinc oxide nanoparticles and zinc sulfate boosts the content of bioactive compounds in habanero peppers. *Plants* 8; Article 254

García-Sánchez F, JL Jifon, M Carvajal, JP Syvertsen (2002). Gas exchange, chlorophyll and nutrient contents in relation to Na^+ and Cl^- accumulation in 'Sunburst' mandarin grafted on different rootstocks. *Plant Sci* 162:705–712

Gopal J, K Iwama (2007). *In vitro* screening of potato against water stress mediated through sorbitol and polyethylene glycol. *Plant Cell Rep* 26:693–700

Gowayed MH, HS Al-Zahrani, SM Metwali (2017). Improving the salinity tolerance in potato (*Solanum tuberosum*) by exogenous application of silicon dioxide nanoparticles. *Intl J Agric Biol* 19:183–192

Gruyer N, M Dorais, C Bastien, N Dassylva, G Triffault-Bouchet (2013) October. Interaction between silver nanoparticles and plant growth. *In: International Symposium on New Technologies for Environment Control, Energy-Saving and Crop Production in Greenhouse and Plant 1037*, pp:795–800. Seoul, Korea

Hammerschmidt R, E Nuckles, J Kuc (1982). Association of enhanced peroxidase activity with induced systemic resistance of cucumber to *Colletotrchum lagenarium*. *Physiol Plant Pathol* 20:73–82

Hancock JF (1999). Strawberries. CABI publishing, New York, USA

Hasegawa PM, RA Bressan, JK Zhu, HJ Bohnert (2000). Plant cellular and molecular responses to high salinity. *Annu Rev Plant Biol* 51:463–499

Hattori T, S Inanaga, H Araki, P An, S Morita, M Luxová, A Lux (2005). Application of silicon enhanced drought tolerance in *Sorghum bicolor*. *Physiol Plant* 123:459–466

Helaly MN, MA El-Metwally, H El-Hoseiny, SA Omar, NI ElSheery (2014). Effect of nanoparticles on biological contamination of *in vitro* cultures and organogenic regeneration of banana. *Aust J Crop Sci* 8:612–624

Hussein EA, AY El-Kerdany, MA Afifi (2017). Effect of drought and salinity stresses on two strawberry cultivars during their regeneration *in vitro*. *Intl J Innov Sci Eng Technol* 4:83–93

Ikeda T, N Suzuxj, M Nakayama, Y Kawakami (2011). The Effects of high temperature and water stress on fruit growth and anthocyanin content of pot-grown strawberry (*Fragaria* ananassa* Duch. cv.'Sachinoka') plants. *Environ Cont Biol* 49:209–215

Jaleel CA, PA Manivannan, A Wahid, M Farooq, HJ Al-Juburi, RM Somasundaram, R Panneerselvam (2009). Drought stress in plants: A review on morphological characteristics and pigments composition. *Intl J Agric Biol* 11:100–105

Khodabin G, Z Tahmasebi-Sarvestani, AHS Rad, SAM Modarres-Sanavy (2020). Effect of drought stress on certain morphological and physiological characteristics of a resistant and a sensitive canola cultivar. *Chem Biodivers* 17; Article 1900399

Klamkowski K, W Treder (2008). Response to drought stress of three strawberry cultivars grown under greenhouse conditions. *J Fruit Ornam Plant Res* 16:179–188

Kruk I, H Aboul-Enein, T Michalska, K Lichszteld, A Kładna (2005). Scavenging of reactive oxygen species by the plant phenols genistein and oleuropein. *Luminescence* 20:81–89

Lichtenthaler HK (1987). Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes. *In: Methods in Enzymology*, Vol. 148, pp:350–382. Colowick SP, NO Kaplan (Eds). Academic Press, London

Mahmoud AWM, EA Abdeldaym, SM Abdelaziz, MB El-Sawy, SA Mottaleb (2020). Synergetic Effects of Zinc, Boron, Silicon, and Zeolite Nanoparticles on Confer Tolerance in Potato Plants Subjected to Salinity. *Agronomy* 10:1–23

Marslin G, CJ Sheeba, G Franklin (2017). Nanoparticles alter secondary metabolism in plants *via* ROS burst. *Front Plant Sci* 8; Article 832

Mozafari AA, F Havas, N Ghaderi (2018). Application of iron nanoparticles and salicylic acid in *in vitro* culture of strawberries (Fragaria× ananassa Duch.) to cope with drought stress. *Plant Cell Tiss Org Cult* 132:511–523

Murashige T, F Skoog (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol Plantarum* 15:473–497

Nair R, SH Varghese, BG Nair, T Maekawa, Y Yoshida, DS Kumar (2010). Nanoparticulate material delivery to plants. *Plant Sci* 179:154–163

Ni X, SS Quisenberry, T Heng-Moss, J Markwell, G Sarath, R Klucas, F Baxendale (2001). Oxidative responses of resistant and susceptible cereal leaves to symptomatic and nonsymptomatic cereal aphid (Hemiptera: Aphididae) feeding. *J Econ Entomol* 94:743–751

Parida AK, AB Das (2005). Salt tolerance and salinity effects on plants: A review. *Ecotoxicol Environ Saf* 60:324–349

Radhi IM, MM Abudl-Hasan (2020). Effect of spraying with proline acid and potassium on chemical traits and yield of strawberry under water stress. *Plant Arch* 20:75–83

Sadasivam S, A Manickam (1991). Amino acids and proteins. *In: biochemical Methods for Agricultural Sciences*, pp: 33–95. Wiley Eastern Ltd., and Tamil Nadu Agricultural University, Coimbtore, India

Saijo Y, E Po-iian Loo (2020). Plant immunity in signal integration between biotic and abiotic stress responses. *New Phytol* 225:87–104

Sardhara K, K Mehta (2018). Effects of abiotic and biotic stress on the plant. *Acad J Bot Sci* 2:5–9

Shatnawi MA, KA Johnson, FR Torpy (2004). *In vitro* propagation and cryostorage of *Syzygium francissi* (Myrtaceae) by the encapsulation-dehydration method. *In Vitro Cell Dev Biol Plant* 40:403–407

Sheikh N, G Hassanzadeh, M Baghestani, B Zabd (2009). Study on the effect of zinc foliar application on the quantitative yield of grain maize under water stress. *Electr J Crop Prod* 2:59–74

Sherwin HW, JM Farrant (1998). Protection mechanisms against excess light in the resurrection plants *Craterostigma wilmsii* and *Xerophyta viscosa*. *Plant Growth Regul* 24:203–210

Snedecor GW, WG Cochran (1989). *Statistical methods* 8th Edition. Ames: Iowa State University Press

Soundararajan P, I Sivanesan, S Jana, BR Jeong (2014). Influence of silicon supplementation on the growth and tolerance to high temperature in *Salvia splendens*. *Hortic Environ Biotechnol* 55:271–279

Sultan MARF, L Hui, LJ Yang, ZH Xian (2012). Assessment of drought tolerance of some *Triticum* L. species through physiological indices. *Czech J Genet Plant Breed* 48:178–184

Sun L, F Song, J Guo, Z Zhu, S Liu, F Liu, X Li (2020). Nano-ZnO-induced drought tolerance is associated with melatonin synthesis and metabolism in maize. *Intl J Mol Sci* 21; Article 782

Tohmaa O, A Esitkena (2011). Response of salt stressed strawberry plant to foliar salicylic acid pre-treatments. *J Plant Nutr* 34:590–599

Turhan E, H Gulen, A Eris (2008). The activity of antioxidative enzymes in three strawberry cultivars related to salt-stress tolerance. *Acta Physiol Plantarum* 30:201–208

Yosefi A, A Akbar Mozafari, T Javadi (2020). Jasmonic acid improved in vitro strawberry (Fragaria× ananassa Duch.) resistance to PEG-induced water stress. *Plant Cell Tiss Org Cult* 142:549–558

Zahedi SM, F Moharrami, S Sarikhani, M Padervand (2020a). Selenium and silica nanostructure-based recovery of strawberry plants subjected to drought stress. *Sci Rep* 10; Article 17672

Zahedi SM, M Karimib, ATJD Silva (2020b). The use of nanotechnology to increase quality and yield of fruit crops. *J Sci Food Agric* 100:25–31

Zhang H, H Kang, C Su, Y Qi, X Liu, J Pu (2018). Genome-wide identification and expression profile analysis of the NAC transcription factor family during abiotic and biotic stress in woodland strawberry. *PLoS One* 13:1–2