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IMPACT OF SILICON *In vitro* NANO PARTICLES APPLICATION ON APPLE ROOTSTOCKS UNDER OSMOTIC STRESS INDUCED BY POLYETHELENE GLYCOL

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ABSTRACT

Callus of three apple (*Malus domestica* Borkh) rootstocks (Balady, MM 106, and MM 111) were subjected to different concentrations of silicon nanoparticles (Si-NPs) at 0, 10 and 100 mgl^{-1} and polyethylene glycol (PEG) at 0, 50 and 100 gl^{-1} to study its potential to drought tolerance. Callus fresh weight (FW) of Balady cv. was increased by 98.7% in PEG-drought stressed conditions after application of high concentration of Si-NPs. Total antioxidants (%), the activity of peroxidase and superoxide dismutase were higher in PEG stressed callus of Balady cv. compared to other cvs. Also, high concentration of free amino acids, proline, reducing sugars and free phenolics were observed in PEG-stressed callus of Balady cv. with increment at high level of Si-NPs. Anatomically, callus cells of Balady cv. were appeared asymmetrically and had lysigenous intercellular spaces under high level of Si-NPs and PEG. The maximum amplicons (14) were amplified using three primers SCoT 26, 66 and 77 was recorded in Balady cv. Application of Si-NPs changed the number of amplicons with different base pair in all apple cvs. under high level of PEG. It can recommend that, application of Si-NPs at 100 mgl^{-1} will increase the tolerance of apple callus to drought, especially in Balady cv., followed by MM106 and MM111 cvs. as a proper drought tolerant rootstock.

INTRODUCTION

Apple (*Malus domestica* Borkh) belongs to Family Rosaceae and native to Southeast of Asia. Anna cultivar was adapted for Egypt conditions where its cold requirements ranged from 300-400 hours. The cultivated area of apple in Egypt reached to 27417 hectare in 2020, with the total productivity of 697936 ton. Anna cv. grafted on more tolerant rootstocks to different environmental and biological conditions, such as Balady, MM 111, and MM 106.

Drought stress adversely impacted the various aspects of plant physiology as photosynthesis, respiration, nutrient absorption, energy metabolism, and enzyme activity which subsequently reduced the growth, development, and productivity of different crops as apple. Drought stress induced osmotic stress, leading to a loss of cell turgor that greatly inhibits cell expansion and division, ultimately slowing plant growth. Water deficits can also induce oxidative stress in plants because of the over accumulation of reactive oxygen species that seriously damages various cellular components, resulting in cell death and metabolic disturbance **(Jia *et al.*, 2021)**.

Osmotic stress induced by PEG reduced the protein synthesis because of inhibition of amino acid incorporation **(Garg *et al.*, 1997)**. Also, proline is one of the most

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prominent osmolytes that accumulate during osmotic adjustment under abiotic stress. Proline is also involved in stabilizing sub-cellular structures, scavenging free radicals, and buffering the cellular redox potential under stress conditions. It can also act as a protein compatible hydro trope **(Srinivas and Balasubramanian, 1995)** to alleviate cytoplasmic acidosis**.** Accumulation of soluble sugars is a common phenomenon for plants growing at high salinity. Sucrose and other simple sugars are effective in stabilizing proteins and in the adjustment of cellular osmotic potential **(Liu and Zhao 2005).**

Plant cells had complex enzymatic and non-enzymatic antioxidant systems to quench different reactive oxygen species (ROS) such as superoxide ($O2^{\bullet -}$) and hydrogen peroxide (H_2O_2) under osmotic stress which had deleterious effect on biological constituents of cell such as chlorophylls, proteins, enzymes, and ribonucleic acids **(Ashraf and Harris, 2004).**

Start Codon Targeted (SCoT) technique was an efficient tool compared to other molecular differentiated tools, due to the longer primer distances and high annealing temperatures **(Collard and Mackill, 2009)**. SCoT procedure was more effective for differentiation among genotypes and treatment than other techniques as inter simple sequence repeat (ISSR) and random amplified polymorphic DNA (RAPD) (**Gorji *et al*., 2011**).

Reduction of fresh water to irrigate different economic crops was the principal problem facing Egypt and world. Therefore, production of more drought tolerant crops such as apple rootstocks is a critical issue. Recently, Si is considered an important element for high plant immunity under different types of stress. Nano silicon particles enhanced the cell wall rigidity, led to reduce plant transpiration, increase disease resistance, and improve plant growth under stresses such as drought and salinity **(Mahmoud *et al.*, 2020)**. Therefore, the research aimed to investigate the impact of Si-NPs application on physiological and molecular parameters of callus of three apple rootstocks (Balady, MM111 and MM106) under drought stress conditions. Drought stress was induced by application of polyethylene glycol (PEG) at 50 and 100 mg/l.

MATERIALS AND METHODS

Explant Sterilization and Callus Induction

This research was done at Prof. Dr. Abd El-Fatah H. Belal Plant Tissue Culture Lab., Fac. of Enviro. Agric. Sci. (FEAS), Arish University, Egypt, during 2018 to 2020. Leave explants of three apple cvs. were sterilized by Clorox solution (30 %) for 20 min., then dipped in ethanol (70%) for 1 min., then immersed in $HgCl_2$ ($1gl^{-1}$) for 5 min. **(Belal *et al*., 2004).** Sterilized explants were transplanted on Murashige and Skoog medium (MS) **Murashige and Skoog (1962)** with addition of sucrose (3%), agar ($7.50\ gl^{-1}$) and pH 5.7- 5.8. Callus induced under different levels of 2,4-D (0.0, 1.0, 2.0 or 4.0 mgl^{-1}) with different concentration of kinetin (Kin) (0.0, 0.1, 0.2, 0.4 mgl^{-1}). Cultures were kept at 25 ± 2^oC under light intensity (2000 Lux) by florescent lamps (Phillips, Egypt). Each treatment composed of 5 replicates and each replicate consisted of 3 jars and each one had four explants. After 40 days the following parameters were recorded:

1. Callus fresh weight (g /jar).

2. Callus increment.

Si-Nanoparticles Application and Drought Stress Induction

For drought stress induction polyethylene glycol (PEG-Sigma 6000) were added to the MS with different concentrations (0.0, 50 or 100 gl^{-1}) combined with Si-NPs at

different levels (0.0, 10 and 100 mgl^{-1}). Nanoparticles (Sigma) with 25 nm size, Si Hydrophilic at 10 and 100 mg/l were used. Equal weight of calli (0.5 g) were used as explants. After 50 days of exposure to drought stress, calli of different treatments were collected and prepared for determine:

Growth parameters

Callus fresh weight (g /jar).

All phytochemicals were estimated using UV/VIS spectrophotometer, PG instrument Ltd, USA.

Proline concentration

Proline was determined by **Bates *et al.* (1973)** at λ 520 nm using fresh callus.

Ethanolic phytochemicals extraction

Phytochemicals in fresh callus was extracted with ethanol 70% according to **Abdel-Rahman *et al.* (1975).** Free phenolics were determined according to **William *et al.* (1965)** at λ 650nm. free amino acids were estimated according to **Rosen (1957)** at λ 570nm. Reducing sugars were determined by Nelson's method described by **Moore (2012)** at λ 540 nm. The total antioxidants were estimated according to **Hatano *et al.* (1988)** as inhibition (%) of DPPH (2,2-diphenyle-1-picryllhydrazyl).

Activity of antioxidants enzymes

Antioxidant enzymes were extracted according to **Urbanek *et al.* (1991).** Peroxidase (POD) activity measured by oxidation of O-dianisidine at λ 430 nm as described by **Urbanek *et al.* (1991).** Superoxide dismutase (SOD) activity was assayed by measuring its ability to inhibit reduction of nitro blue tetrazolium (NBT) at λ 560 nm as explain by **Beauchamp and Fridovich (1971).** Activity determined as units/100g protein. min.

Soluble protein concentration

Soluble protein concentration determined according to **Bradford (1976)** using Coomassie brilliant blue G-250 (Sigma).

Histological investigation:

For longitudinal sections (15 µm thick), callus was fixed in formalin acetic acid (FAA), then dehydrated using ethanol series and cleared with ethanol-xylene. Samples were embedded in paraffin wax at 45-55ºC **(Willey, 1971)**. The fixed sections were stained with Safranin O-Fast-green double stain. Observation and photomicrographs were done using research microscope (LEICA DM500) with digital camera (LEICA ICC50).

SCoT analysis

The DNA of callus was participated by CTAB buffer according to **Doyle and Doyle (1973)**. The specific amplicon of DNA was amplified using automated thermal cycle (model Techno 512). One cycle arranged as 95ºC for 5 min, followed by 40 cycles of 30 sec. at 95ºC, 30 sec. at 57ºC (temperature of annealing), and 30 sec. at 72ºC. Three different primers with sequence were SCoT 26, 5'ACCATGGC TACCACCGTC' 3; SCoT 66, 5' ACCATG GCTACCAGCGAG'3; SCoT77,5'CCAT GGCTACCACTACCC'3. DNA ladder marker was used as standard DNA with molecular weights of 100 to1500 bp. The run was performed for about 30 min at 80 V in mini submarine gel BioRad. The polymorphism percentage was calculated, according to **Patra *et al.* (2008)**.

Experimental Design and Statistical Analysis

Five replicates and each replicate contained 3 explants for each treatment at a completely randomized design (CRD) were used. Data were tested by using Co-STAT software V.6.13 (Cohort software, Berkeley, CA 94701). Mean values of treatments were differentiated by using least significant range (Duncan's multiple range testes) at 0.01% level probability **(Duncan, 1975).**

RESULTS AND DISCUSSION

Callus Fresh Weight (g) as Affected by Si-Nps Application under Drought Stress Conditions

As shown in Table 1and Fig. 1, cultivars, drought, and Si-NPs had significant effect on callus FW in all apple cultivars under study. The highest significant value (5.466 g) of callus FW was shown in Balady cv. treated by 0 gl^{-1} PEG + 100 mgl^{-1} Si-NPs, followed by MM 106 cv. (4.10 g), then MM 111 cv. (3.80 g). Results obvious that callus of Balady cv. was the most tolerant to drought at high level of PEG (100 gl^{-1}) after application of Si-NPs. FW was increased by 98.7% after application of high concentration of Si-NPs.

Callus color was white under all treatments, which reflect the conserved role of Si-NPs on cells under drought conditions. The results reported herein were agreed with **Danial *et al.* (2015)** who found that PEG had no effect on callus initiation in apple, but callus weight was decreased as PEG increment in MS medium. However, **Al-Mayahi (2016)** showed that Si application improved growth of *in vitro* date palm under drought stress. Also, **Avestan *et al.* (2016)** found high proliferation (%) in MM106 cv. of apple after application of 100 mg/l of silicon oxide. **Mahmoud *et al.* (2020)** observed high shoot growth and chlorophyll content after addition of SiO_2 NPs of *in vitro* banana under water deficit, induced by PEG-8000).

Biochemical Parameters

Total antioxidants (%), peroxidase (POD) and superoxide dismutase (SOD)

Drought, Si-NPs, and cultivars had a significant effect on total antioxidants (%), POD and SOD activity in all cvs. as shown in Table 2. The maximum significant value of total antioxidants (30.59), POD (7.76) and SOD (1.570) was shown in Balady cv. treated with 100 gl^{-1} PEG + 100 mgl^{-1} Si-NPs, followed by MM 106 cv. which gave 30.56% of total antioxidants, POD (3.05) and SOD (0.836) activity, then MM 111 cv. which gave (30.53%) of total antioxidants, (1.32) of POD and (0.706) of SOD activity. Meanwhile, MM 111 cv. showed the minimum value for each total antioxidants (9.46%), POD (0.09) and SOD (0.050) activity was recorded with MM 111 cv. treated by 0 gl^{-1} PEG + 0 mgl^{-1} Si-NPs, followed by MM 106 cv. which gave 9.48% of total antioxidants, POD (0.08) and SOD (0.056) activity, then Balady cv. which gave (9.51%) of total antioxidants, (0.09) of POD and (0.063) of SOD activity. These findings obvious that, Balady cv. more tolerant to drought stress followed by MM 106 cv. but MM111 cv. showed more sensitivity to drought stress. These findings were in harmony with **Wang *et al.* (2020)** who showed high activity of SOD in apple leaves under drought stress compared to unstressed one. Also, **Jiroutova *et al.* (2021)** found high activity of SOD in apple explants under drought stress induced by PEG at 50 gl^{-1}.

Free amino acids, proline, and soluble protein (mg/100g FW)

Drought, Si-NPs, and cultivars also, had significant effect on free amino acids, proline, and soluble protein concentrations (mg/100g FW) in all cvs. as shown in Table 2. The highest significant values of free amino acids (20.65) and proline (20.29) mg/100g FW were recorded in Balady cv. under 100 gl^{-1} PEG + 100 mgl^{-1} Si-NPs, followed by MM 106 cv. which gave 17.68 and 17.73 of free amino acids and proline, respectively then MM 111 cv. which had 12.80 and 15.87 proline and free amino acids, respectively. The maximum value of soluble protein (162.73) mg/100g FW was recorded in Balady cv. which exposed to 0 gl^{-1} + 100 mgl^{-1} Si-NPs, followed by MM 106 cv. which had 162.62 mg/100g FW, then MM 111 cv. (162.52) mg/100g FW. Results reported herein were coordinated with **Rajabpoor *et al.* (2014)** who showed

Table 1. Callus fresh weight (g) and increment or decrement (%) of callus weight compared to control as affected by S-NPs application in three apple cvs. under drought stress induced by PEG

	Treatment		Callus fresh weight (g)	Increment or decrement compared to control (%)
Balady	PEG stress 0 gl^{-1} (control)	Nano-Si 0 mgl^{-1}	4.10 ab	0.0
		Nano- Si 10 mgl^{-1}	3.40 a-d	-17.07
		Nano- Si 100 mgl^{-1}	5.466 a	33.31
	PEG stress 50gl^{-1}	Nano-Si 0 mgl^{-1}	1.60 bcd	0.0
		Nano- Si 10 mgl^{-1}	2.80 bcd	75.0
		Nano- Si 100 mgl^{-1}	1.60 bcd	0.0
	PEG stress 100 gl^{-1}	Nano-Si 0 mgl^{-1}	1.50 cd	0.0
		Nano- Si 10 mgl^{-1}	1.80 bcd	20.0
		Nano- Si 100 mgl^{-1}	2.98 bcd	98.7
MM 106	PEG stress 0 gl^{-1} (control)	Nano-Si 0 mgl^{-1}	2.98 bcd	0.00
		Nano- Si 10 mgl^{-1}	2.20 bcd	-26.17
		Nano- Si 100 mgl^{-1}	4.10 ab	37.6
	PEG stress 50gl^{-1}	Nano-Si 0 mgl^{-1}	1.40 cd	0.0
		Nano- Si 10 mgl^{-1}	2.60 bcd	85.7
		Nano- Si 100 mgl^{-1}	1.20 d	-14.3
	PEG stress 100 gl^{-1}	Nano-Si 0 mgl^{-1}	1.28 d	0.0
		Nano- Si 10 mgl^{-1}	1.40 cd	9.4
		Nano- Si 100 mgl^{-1}	1.40 d	9.4
MM 111	PEG stress 0 gl^{-1} (control)	Nano-Si 0 mgl^{-1}	3.04 bcd	0.0
		Nano- Si 10 mgl^{-1}	1.80 bcd	-40.8
		Nano- Si 100 mgl^{-1}	3.80 abc	25.0
	PEG stress 50gl^{-1}	Nano-Si 0 mgl^{-1}	1.00 d	0.0
		Nano- Si 10 mgl^{-1}	1.80 bcd	80.0
		Nano- Si 100 mgl^{-1}	1.20 d	20.0
	PEG stress 100 gl^{-1}	Nano-Si 0 mgl^{-1}	1.20 d	0.0
		Nano- Si 10 mgl^{-1}	1.60 bcd	33.3
		Nano- Si 100 mgl^{-1}	1.20 cd	0.0

- Mean values of treatments were differentiated by using Least Significant Range (Duncan's multiple range test) at 0.01 level probability

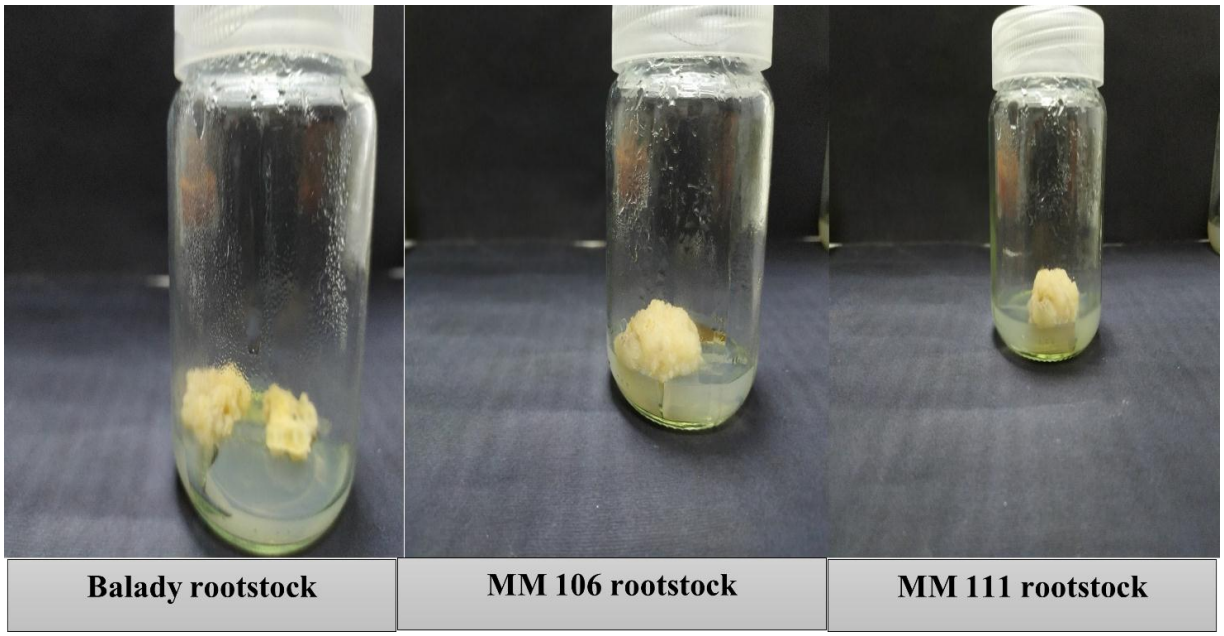

Fig. 1. 40 days-old callus in three apple rootstocks

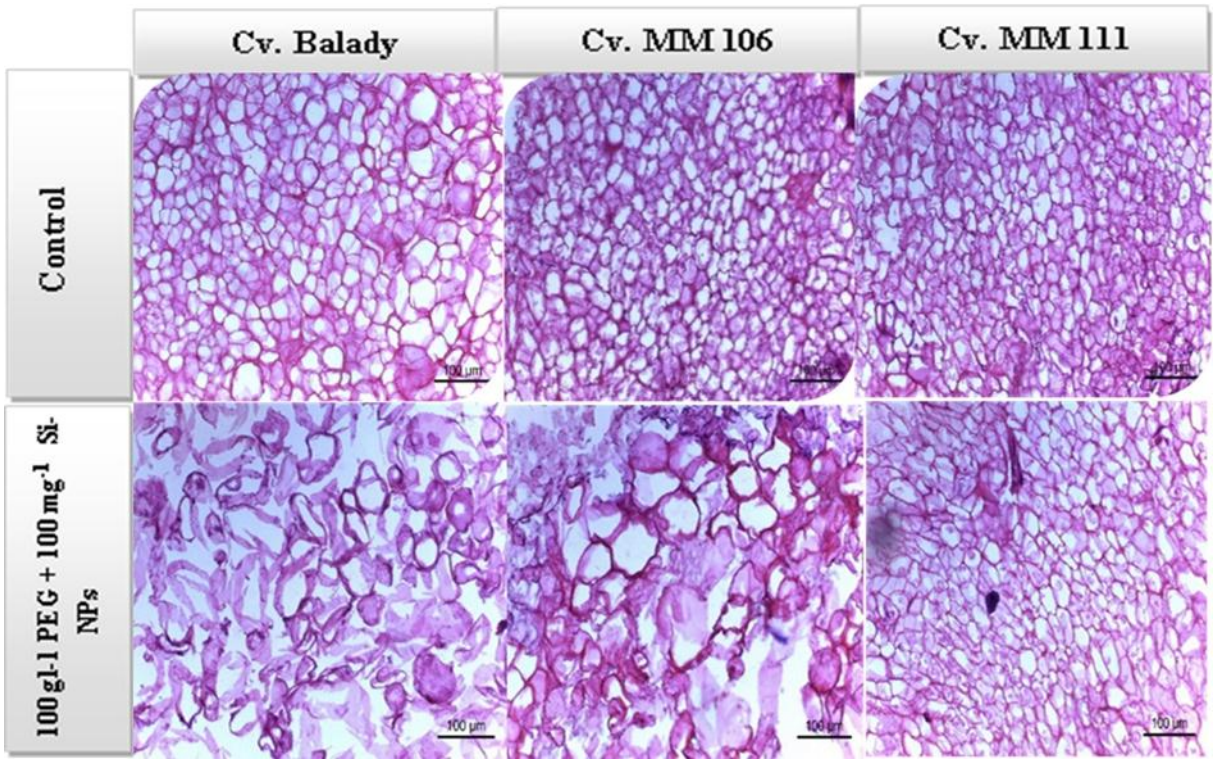

Fig. 2. Histological modifications of callus cells as affected by Si-NPs application in three apple cvs. under PEG-induced drought stress

Table 2. Total antioxidants (%), peroxidase (POD), superoxide dismutase (SOD), free amino acids, proline, soluble protein, reducing sugars and free phenolics (mg/100g FW) as affected by Si-NPs addition in three apple cvs. under PEG as osmotic stress

Treatment			Total antioxidants (%)	POD units/100g protein.min	SOD units/100g protein.min	Free amino acids	Proline	Soluble protein	Reducing sugars	Free phenolics
Balady	PEG stress 0 gl^{-1}(control)	Nano-Si 0 mgl^{-1}	9.51 c	0.09 c	0.063 b	0.99 o	2.45 g	154.86 ab	7.27 w	90.98 x
		Nano- Si 10 mgl^{-1}	10.30 c	0.10 c	0.083 b	3.96 n	2.73 g	159.47 a	8.31 t	100.95 v
		Nano- Si 100 mgl^{-1}	13.56 bc	0.11 c	0.186 b	4.11 lmn	2.93 g	162.73 a	8.80 q	137.14 s
	PEG stress 50gl^{-1}	Nano-Si 0 mgl^{-1}	16.31 bc	0.17 c	0.166 b	4.91 jkl	3.16fg	147.13 ab	11.22 o	155.71 p
		Nano- Si 10 mgl^{-1}	18.79 abc	0.28 c	0.236 b	5.50 ij	3.33fg	151.93 ab	13.29 l	181.90m
		Nano- Si 100 mgl^{-1}	19.10 abc	0.34 c	0.206 b	6.89 g	3.65fg	153.25 bc	14.46 i	195.71 j
	PEG stress 100 gl^{-1}	Nano-Si 0 mgl^{-1}	20.82 abc	0.44 c	0.283 b	8.31 fg	6.47 e	102.61 d	15.04 g	431.33 g
		Nano- Si 10 mgl^{-1}	24.38 ab	0.94 c	0.386 b	11.73 d	12.37c	125.79 c	21.75 d	644.28 d
		Nano- Si 100 mgl^{-1}	30.59 a	7.76 a	1.570 a	20.65 a	20.29a	135.44 bc	32.07 a	836.19 a
MM 106	PEG stress 0 gl^{-1}(control)	Nano-Si 0 mgl^{-1}	9.48 c	0.08 c	0.056 b	0.96 o	2.43 g	154.82 ab	5.80 x	90.95 x
		Nano- Si 10 mgl^{-1}	10.28 c	0.10 c	0.080 b	1.31 o	2.62 g	159.44 a	8.23 u	100.47 v
		Nano- Si 100 mgl^{-1}	13.53 bc	0.11 c	0.180 b	4.11 lmn	2.88 g	162.62 a	8.68 r	116.19 t
	PEG stress 50gl^{-1}	Nano-Si 0 mgl^{-1}	16.30 bc	0.17 c	0.156 b	4.70 j-m	3.08 g	147.10 ab	11.22 o	154.76 q
		Nano- Si 10 mgl^{-1}	18.71 abc	0.20 c	0.210 b	5.21 ijk	3.23fg	151.89 ab	13.02 m	175.71 n
		Nano- Si 100 mgl^{-1}	19.02 abc	0.32 c	0.204 b	6.63 gh	3.62fg	153.21 bc	14.33 j	192.85 k
	PEG stress 100 gl^{-1}	Nano-Si 0 mgl^{-1}	20.79 abc	0.42 c	0.280 b	8.02 f	4.62 f	102.50 d	14.59 h	242.85 h
		Nano- Si 10 mgl^{-1}	24.30 ab	0.68 c	0.380 b	10.41 e	10.1d	125.78 c	19.68 e	589.04 e
		Nano- Si 100 mgl^{-1}	30.56 a	3.05 b	0.836 b	17.68 b	17.7b	135.40 bc	25.24 b	793.81 b
MM 111	PEG stress 0 gl^{-1}(control)	Nano-Si 0 mgl^{-1}	9.46 c	0.03 c	0.050 b	0.46 o	2.42 g	154.79 ab	3.91 y	67.14 y
		Nano- Si 10 mgl^{-1}	10.25 c	0.09 b	0.078 b	1.21 o	2.48 g	159.40 a	8.12 v	96.19 w
		Nano- Si 100 mgl^{-1}	13.44 bc	0.10 c	0.156 b	3.86 mn	2.76 g	162.52 a	8.43 s	115.23 u
	PEG stress 50gl^{-1}	Nano-Si 0 mgl^{-1}	16.23 bc	0.14 c	0.153 b	4.41 k-n	2.96 g	147.06 ab	10.31 p	152.85 r
		Nano- Si 10 mgl^{-1}	18.72 abc	0.18 c	0.200 b	5.16 ijk	3.19fg	151.75 ab	11.53 n	171.90 o
		Nano- Si 100 mgl^{-1}	19.02 abc	0.30 c	0.183 b	5.92 hi	3.36fg	153.08 bc	14.10 k	183.33 l
	PEG stress 100 gl^{-1}	Nano-Si 0 mgl^{-1}	20.76 abc	0.39 c	0.276 b	7.05 g	3.85fg	102.39 d	14.58 h	224.76 i
		Nano- Si 10 mgl^{-1}	24.30 ab	0.55 c	0.297 b	8.90 f	7.35 e	125.67 c	17.15 f	440.00 f
		Nano- Si 100 mgl^{-1}	30.53 a	1.32bc	0.706 b	15.87 c	12.80c	135.26 bc	24.77 c	680.00 c

- Mean values of treatments were differentiated by using Least Significant Range (Duncan's multiple range test) at 0.01 level probability

that soluble protein content was significantly declined in eight wild almond species under high drought stress. However, application of Si-NPs under drought stress reduced the osmotic potential of cell sap of *Juglans regia* by accumulation of metabolism-compatible solutes such as proline, glycine betaine, sorbitol, and soluble carbohydrates **(Karimi *et al.*, 2018)**. Also, **Yang *et al.* (2021)** remarked that drought stress significantly reduced the photosynthetic productivity, and seriously damaged the physiological metabolism of leaves of 'Yulu Xiang' Pear.

Reducing sugars and free phenolics

Drought, Si-NPs, and cultivars had significant effect on reducing sugars and free phenolics in all cvs. as shown in Table 2 and Fig 2. The highest significant value for each of reducing sugars (32.07 mg/100g FW) and free phenolics (836.19 mg/100g FW) was achieved with 100 gl^{-1} PEG + 100 mgl^{-1} Si-NPs with Balady cv., followed by MM 106 cv. which gave (25.24 mg/100g FW) of reducing sugars and (793.81 mg/ 100g FW) of free phenolics, then MM 111 cv. which gave (24.77 mg/100g FW) of reducing sugars and (680.00 mg/100g FW) of free phenolics. Our results were coordinated with **Mahmoud *et al.* (2017)** who cleared that *in vitro* banana (*Musa* spp.) plantlets had high concentration of free phenolics after application of PEG at 3% compared to control one. The beneficial role of Si-NPs may be maintaining the plant integrity as shown in banana cv. 'Grand Nain' under abiotic stress **(Mahmoud *et al.*, 2020)**. **Rajabpoor *et al.* (2014)** found that sugars content was increased in eight wild almond species under more negative osmotic potential but decreased at -1.2 MPa.

Molecular response of cultivars to high concentration of Si-NPs and PEG

Table 3 and Fig. 2 show that apple cultivars were differed at genetic level according to the overall amplicons which amplified after using three different SCoT primers, 66, 77 and 26. Although overall bands were decreased from 14 to 11 bands in Balady cv., it increased from 5 to 10 bands in MM106 cv. and decreased from 7 to 0 bands MM111 cv. after application of Si-NPs to PEG-stressed callus. All primers were efficient for differentiation among cultivars and treatment due to the high polymorphic bands as 100%. Addition of Si-NPs to PEG-stressed callus led to presence or disappear specific bands. Exposure callus to both drought and Si-NPs changed the genetic amplicon which amplified by different SCoT primers. Bands with 300 bp amplified by ScoT 66, 350 bp by SCoT 77 and both 300,400 bp by SCoT 26 which found in unstressed callus of Balady cv. were absent in drought stressed one after application of Si-NPs. However, bands with 500 bp produced by SCoT 66 and bands with 100 to 300 bp formed by SCoT 77, bands with 400 bp by SCoT 26 were present because of application of Si-NPs to PEG-stressed callus of MM106 cv. Also, bands with 200 to 300 bp formed by SCoT 77 and bands with 150 to 300 bp formed by SCoT 26 was absent after application of Si-NPs to PEG-stressed callus of MM111 cv.

Histological modifications because of high Si-NPs and PEG application

Application of Si-NPs to PEG-stressed callus of MM106 cv. gave the maximum polar (P) and equatorial (E) diameter of cells (96 and 82 µm, respectively) compared to other treatments (Table 4). Addition of Si-NP at 100 mgl^{-1} to osmotic-stressed calli of MM 111 cv. gave the high P/E ratio, with oblate shape of cells **Lyndon (1990**), cited that cell with elongated shape was more rapidly differentiate into vascular tissues compared to spherical one**.** Cells were asymmetric and had lysigenous intercellular spaces after application of Si-NPs to PEG stressed calli in both Balady and MM106 cvs.. Exposure calli of all three cultivars to PEG treatment with Si-NPs was suppressed the cell division. The minimum reduction of cell division (0.57 time) was recoded in MM111 cv. Drought or osmotic

Table 3. Variations among NPs treated and control callus of apple cultivars in the number of bands using three SCoT primers under PEG stress.

			SCoT 66							SCoT 77						SCoT 26						
			MW (bp)							MW (bp)						MW (bp)						
			500	300	250	200	150	100	Total bands	350	300	250	200	150	Total bands	400	300	200	150	100	Total bands	Overall bands
Cultivar	**PEG (100 g/l)**	**Si**																				
Balady	**0.0**		-	+	+	+	+	+	**5**	+	+	+	+	+	**5**	+	+	+	+	-	**4**	**14**
	100	**100**	+	-	+	+	+	+	**5**	-	+	+	+	+	**4**	-	-	+	+	-	**2**	**11**
MM 106	**0.0**		-	-	-	-	+	+	**2**	-	-	-	-	-	**0**	-	+	+	+	-	**3**	**5**
	100	**100**	-	-	-	-	+	+	**2**	-	+	+	+	+	**4**	+	+	+	+	-	**4**	**10**
MM 111	**0.0**		-	-	-	+	-	-	**1**	-	+	+	+	-	**3**	-	+	+	+	-	**3**	**7**
	100	**100**	-	-	-	-	-	-	**0**	-	-	-	-	-	**0**	-	-	-	-	-	**0**	**0**
Monomorphic bands %					**0.0%**							**0.0%**						**0.0%**				
Polymorphic bands %					**100%**							**100%**						**100%**				

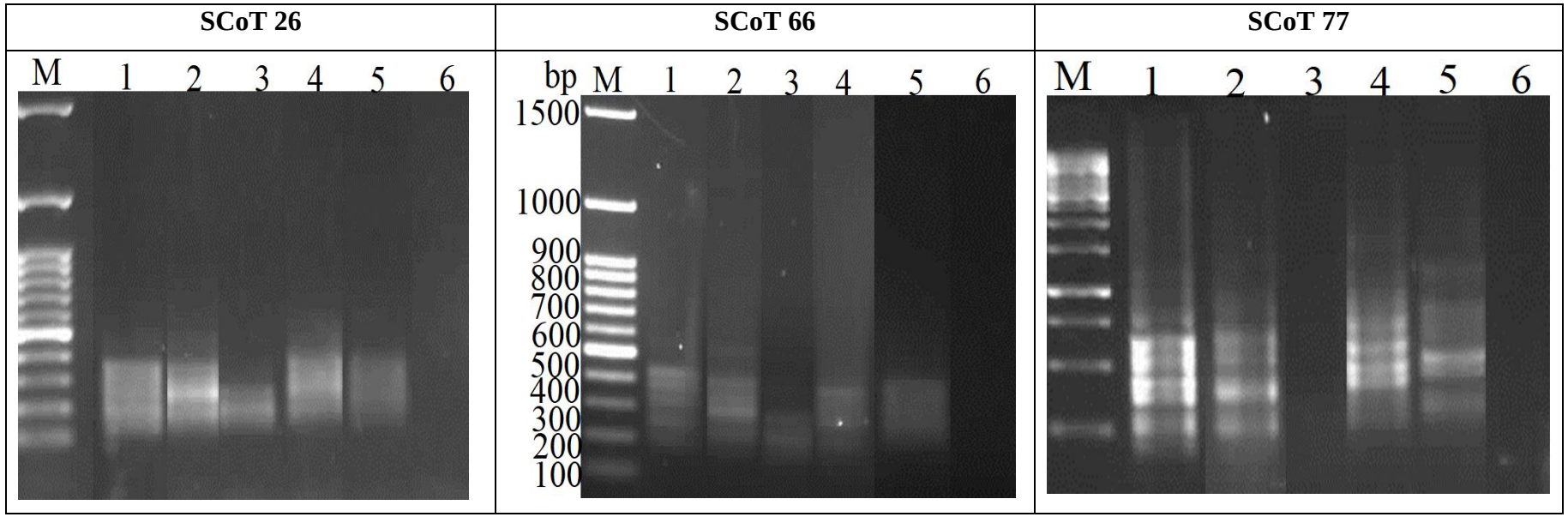

Fig. 2. SCoT amplification profile of Si-NPs treated callus and control of three apple cultivars produced by 3 different primers as affected by drought stress. M, marker; 1, 2 Balady cv.; 3,4 MM106 cv.; 5,6 MM111 cv.; 1,3,5 control; 2, 4, 6 Si-NPs + 100 gl^{-1} of PEG.

Table 4. Histological variations among Si-NPs treated and control callus of apple cultivars under PEG stress for 90 days

Cultivar	PEG (mg/l)	Si-NPS	Average (µm) of the		P/E ratio	Cell		Intercellular spaces	average		
			Polar (P) diameter of cell maximum	equatorial (E) diameter of cell maximum		Shape	Symmetry		number of cells / mm^2	number of cells formed/day	Times of reduction
Balady	0.0	0.00	52 d	40 c	1.3 :1	Prolate	multiseriate	shizogenous	724 c	8.0 c	4.6
	100	100	91 b	62 b	1.5 : 1	semi-prolate	asymmetric	lysigenous	130 d	1.4 d	
MM 106	0.0	0.00	49 d	39 cd	1.3 :1	Prolate	multiseriate	shizogenous	800 b	8.9 b	7.1
	100	100	96 a	82 a	1.2 : 1	semi-spheroid	asymmetric	lysigenous	99 e	1.1 e	
MM 111	0.0	0.00	39 e	28 e	1.4 : 1	semi-prolate	multiseriate	shizogenous	1253 a	13.9 a	0.57
	100	100	69 c	35 d	2 :1	Oblate	multiseriate	shizogenous	800 b	8.9 b	

stress had negative effects on cell divisions due to formation of different reactive oxygen species **(Zhu, 2002)**. Calli of MM111 cv. under control treatment gave the highest number of cells/mm^2 and number of cells formed/day (1253 and 13.9, respectively) compared to other treatments.

Conclusion

Balady cv. was more tolerant to drought stress by showing the highest values of total antioxidants, POD, SOD, free amino acids, proline, reducing sugars and free phenolics content, followed by MM 106 cv. but MM111 cv. showed more sensitivity to stress. Using of Si-NPs at (100 mgl^{-1}) increased callus growth and tolerance under drought stress.

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الملخص العربي

تأثير إضافة جزيئات النانوسليكون على أصول التفاح تحت الاجهاد الأسموزي المستحث بالبولي ايثيلين جليكول معملياً

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تم تعريض كالس ثلاثة أصول للتفاح هي (البلدي – MM 106 – MM 111) لتركيزات مختلفة من جزيئات النانو سيليكون هي (0, 10 and 100 mgl^{-1}) والاجهاد المائى المحفزبالبولى إيثيلين جليكول (0, 50 and 100 mgl^{-1})، ولدراسة قدرتها على تحمل الجفاف وتأثير المعاملات على نمو الكالس والمكونات البيوكيميائية له. زاد الوزن الغض لكالس الصنف البلدي بنسبة 98.7% تحت ظروف اجهاد الجفاف بالبولي ايثيلين جليكول بعد اضافة النانو سيليكون بتركيز (100 mgl^{-1}) واجهاد مائي مستحث بالبولي إيثيلين جليكول بتركيز (100 mgl^{-1}). فى نفس الوقت اظهرت نفس الظروف على الكالس أعلى قيم لمضادات الأكسدة الكلية، وأعلى نشاط للبيروكسيديز، السوبر أوكسيد ديسميوتيز، واعلى تركيزللأحماض الأمينية الحرة، البرولين، السكريات المختزلة والفينولات الحرة مقارنة بباقي الأصناف. من الناحية التشريحية اظهر الكالس خلايا غير متناسقة ومسافات بينية انقراضية دليلا على الاسراع في التميز النسيجي. كما سجل أعلى وجود لـ 14 حزمة وراثية باستخدام 3 أنواع مختلفة من البوادئ الوراثية. كما أدى إضافة جزيئات النانو سليكون إلى ظهور أو اختفاء حزم وراثية معينة تحت ظروف الجفاف. يمكن التوصية بإضافة النانوسليكون بتركيز 100 ملجرام/لتر إلى الكالس خاصة مع الصنف البلدى لزيادة قدرتها على تحمل الجفاف.

الكلمات الاسترشادية: التفاح، زراعة الأنسجة، بولي إيثيلين جليكول، النانوسليكون، ScoT.

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