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IMPROVING HEAT STRESS TOLERANCE IN POTATO (*SOLANUM TUBEROSUM*) L.

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Heat stress is one of the major abiotic stresses affecting potato growth and productivity in semi-arid and growing zones. Using new biotechnology approaches and application of nanomaterials could improve the productivity and profitability of potato. Silicon nanoparticles (SiO_2 NPs) responsible for improvements lead finally to the enhancement of plant growth and productivity under salinity, drought, and heavy metal stresses. In this study, the effectiveness of SiO_2 NPs at 5, 10 and 20 ppm under different levels of heat of 25, 35 and 45°C on growth and biochemical parameters was *in vitro* studied, for four potato cultivars. Results referred to the superiority of Agria, which was positively and significantly affected by SiO_2 NP treatments followed by Hermes, and Cara. On the other hand, there was no significant effect of SiO_2 NPs on Diamond cv, under different heat levels. At 45°C, Agria cv. produced the highest value of microtubers weight and length at 0, 5, 10 and 20 ppm of SiO_2 NPs, followed by Diamond with SiO_2 NPs of 5 and 20 ppm. The heighest vegetative characteristics of Agria were 14.67 cm for shoot length, 7.00 cm for shoots/explant, 5 cm for root length, and 10.33 for roots number/ explant, at 35°C with 10 ppm SiO_2 NPs. About harvesting, for the same cultivar, microtubers weight was 0.89, 0.79 and 0.93 g at 45°C, without significant differences among SiO_2 NPs. Microtubers number was 5.33 by 5 ppm at 25°C. Microtubers length was 1.43 cm with 5 and10 ppm SiO_2 NPs at 45°C. For microtubers diameter, there was no significant effect among SiO_2 NPs at all heat levels. Also, glutathione higher value was obtained by 5 ppm at 45°C. Concerning lipid peroxidation level, the lowest level of the toxic product malondialdehyde (MDA) was obtained by 0 ppm, increased in other treatments at 25°C. At 35°C, the minimum level of MDA was recorded by 20 ppm and in general the highest temperature applied (45°C) increased MDA accumulation.

Keywords: potato, heat stress, microtubers, nanomaterials, SiO_2 NPs, glutathione, lipid peroxidation

INTRODUCTION

Potato (*Solanum tuberosum* L.) is the most important tuber crop and fourth main food crop in the world after maize, rice and wheat, which grows in about 150 countries and plays a vital role in the global food system. Potato ranks second in the list of the Egyptian agricultural exports after cotton, of which 171.012 metric tons were export to Europe and some Arab countries. It is cultivated in over 100 countries, and the global production of potatoes was estimated to be 370 million tons (FAOSTAT, 2019), feeding over a billion people worldwide (Moeinil et al., 2011 and Othman et al., 2016). Potato is a cool weather crop but now becomes a worldwide crop grown under many climatic conditions. The highest yield was obtained in regions with moderate climate, such as northern United States and northwestern Europe (Levy and Veilleux, 2007). Tuberization is optimal at short days, average temperatures in the 15-20°C range, tuber formation were declines above this range. Temperature above the optimal causes reduction in tuber yield and productivity, although moderately elevated temperatures of 20-25°C may enhance potato vegetative growth. High temperatures during the growing season affect its development and may lead to a drastic reduction in economic yield (Rykaczewska, 2015; Tang et al., 2018 and Momčilović, 2019). High temperatures decrease harvest index and thus, tuber weight and increase leaf dry matter (Hancock et al., 2014). Heat stress also causes tubers cracking, hollow heart, secondary growth, malformations, induces heat necrosis in the tuber flesh and stimulates conversion of starch to primary sugar leading to dark French fries (Minhas, 2012 and Wang-Pruski and Schofield, 2012).

In vitro propagation of potato is generally used to produce new lines, store germplasm, incubate microtubers or minitubers which thus are easy to store and transport (Uranbey, 2017). Microtubers are magic solution to insure healthy and free-virus asexual seeds (Islam et al., 2017 and Mohapatra and Batra, 2017). Multifactors control formation of microtubers like sucrose, temperature, growth regulators and genotypes (Emara et al., 2017 and Hossain et al., 2017). Microtubers formation is effective at sucrose concentrations ranged between 60-90 g L^{-1} and the low temperature (18°C) (Khalil et al., 2017).

Nanotechnology is widely used in plant biotechnology and has a wide range of applications, such as nutrient consumption, plant germination, plant growth, and stress tolerance in agriculture. Nanoparticles (NPs) are increasingly being used to trigger the production of beneficial secondary metabolites from natural sources. Considering their diverse impacts on live cells, their potential microscopic size, vast surface area, reactivity, and strong propensity to penetrate the plasma membrane make them excellent for biological applications (Sharada et al*.,* 2018). However, in terms of callus culture, the relevance of NPs in plant secondary metabolism remained unclear (Wang et al*.,* 2016). Several studies reported the positive influence of NPs application on different plants' growth and development, particularly at low concentrations under different abiotic stress conditions (Mahdi, 2017; Hassan et al., 2018; Farroh et al*.,* 2020; Mahdi et al*.,* 2020 and El-Saber et al., 2021). The beneficial effects of silicon and its role for plants are well established. Silicon NPs have unique physiological properties that enable them to penetrate plants and alter their metabolic processes. Silicon NPs mesoporous structure makes them ideal nano-carriers for a variety of compounds that might be useful in agriculture and as elicitors in the tissue culture field (Rastogi et al., 2019). Silicon is also considered somewhere between an essential and non-essential element for plants, as it is not required for the survival of most plants, but plants benefit and are better adapted to different environmental stress conditions in the presence of silicon (Luyckx et al*.,* 2017). Silicon has also been observed to be used by plants to strengthen their cell walls. SiO_2 NPs can also reduce the transpiration rate of the plant, rendering it more resistant to limited water supply (drought), high temperature and humidity (Adisa et al., 2019; Rastogi et al., 2019; Rajput et al., 2021 and Verma et al., 2021).

Heat stress is one of the most significant environmental conditions that can harm agricultural plants, impairing a variety of physiological and biochemical processes (Paradiso et al*.,* 2020). Heat stress also causes oxidative stress in plants by accelerating the production of reactive oxygen species (ROS) such as singlet oxygen, superoxide anion, hydrogen peroxide and hydroxyl radical (Hasanuzzaman et al., 2020). Autocatalytic peroxidation of membrane lipids and pigments, as well as changes in membrane permeability and functions, are the principal consequences of ROS (Xu et al., 2008). Lipid peroxidation has long been employed as a marker of free radical damage to cell membranes under stressful situations. The main and well-studied product of Poly Unsaturated Fatty Acid (PUFA) peroxidation is malondialdehyde (MDA). This aldehyde is a very harmful substance that deserves to be thought of as more than a lipid peroxidation marker (Rio et al., 2005). Different aldehydes are generated when PUFAs in bio-membranes are peroxidized, including the extremely reactive aldehyde MDA. It is mostly produced in chloroplasts (Yamauchi et al*.,* 2008).

Therefore, the objective of this study was to investigate the effectiveness of SiO_2 NPs in elevating the negative effect of heat stress on micropropagation and microtubers formation of four potato varieties (Agria, Cara, Diamond and Hermes) and some biochemical characters.

MATERIALS AND METHODS

The present study was carried out during the period from 2018 to 2020 at the Tissue Culture Unit, Genetic Resources Department, Desert Research Centre, Cairo, Egypt and National Gene Bank, Agricultural Research Centre, Giza, Egypt.

1. *In Vitro* Preparation

1.1. Culture medium

Murashige and Skoog (MS) micro and macro-elements medium (1962) were used in the present study (Sigma-Aldrich, Germany). The pH of the medium was adjusted to 5.7 using 1.0 M HCl or 1.0 M NaOH. Phytagel at 3% (w/v) was added after pH adjustment. Media were dispensed into glass jars and sterilized for 20 min at 121°C (15-psi steam pressure with an extended cycle time of 30 min).

1.2. Explants

Tubers were brushed and washed under running water to exclude mud, dirties, and kept in closed paper bags at 24°C until small sprouts appeared. The excised eye buds (sprouts) were rinsed in distilled water, dipped in 70% ethanol for one min and stirred in 0.1% mercuric chloride for 10 min. To overcome phenols formation, materials were put in an antioxidant-sterilized solution (100 mg L^{-1} ascorbic acid and 150 mg L^{-1} citric acid) for 10 min. Finally, sprouts were rinsed with sterile distilled water three times.

1.3. Establishment of *in vitro* cultures

Sterilized sprout meristems 0.3-0.5 mm, one meristem, were placed in culture tubes (25x150 mm) containing 10 ml of solid starting MS medium supplemented with 2 mg L^{-1} D-calcium pantothenate, 0.1 mg L^{-1} gibberellic acid and 30 g L^{-1} sucrose. The medium was solidified with 3.0 g L^{-1} phytagel. Combination between BAP and NAA were used for four cultivars mass micropropagation (Sallam, 2014). Cultures were incubated at 20±2°C in a perfect darkness condition for 24 h then under 16 h (2000 lux, daily fluorescent tubes) for 2-4 weeks to produce *in vitro* virus-free-plantlet.

2. *In Vitro* Heat Stress Experiment

2.2. *In vitro* propagation under heat stress

In vitro multiplication of virus-free-stock plants were obtained through meristem culture using nodal cuttings with 1-2 nodes each on solid MS medium with D-calcium pantothenate 2 mg L^{-1}, and 30 g L^{-1} sucrose and medium was gelled using 3% phytagel (Sallam, 2014). Media supplemented with different levels of SiO_2 NPs (0.0, 5.0, 10.0, and 20.0 ppm) combined with three degrees of heat (25, 35 and 45°C) in 12 treatments for each cultivar. Two plantlets (2 nodes) in each tube containing 12 ml medium and five culture tubes were considered as a treatment for the four cultivars. Cultures were incubated for 3-4 weeks at 20±2°C under a 16 h photoperiod using cool white fluorescent lights (50-60 µE/m2/s light intensity). Shoot number, shoot length (cm), root number and root length (cm) were recorded for each micropropagule.

2.2. *In vitro* microtuberization under heat stress

The microtubers induction medium was MS basal nutrients supplemented with sucrose at 60 g L^{-1}, gelled with 3% phytagel (Sallam, 2014). Media supplemented with different levels of SiO_2 NPs (0.0, 5.0, 10.0, and 20.0 ppm) combined with three degrees of heat (25, 35, and 45°C) in 12 treatments /cultivar were tested. Two plantlets (2 nodes) in each tube containing 12 ml medium and five culture tubes were considered as a treatment for the four cultivars. Cultures were incubated for 10 days at 20±2°C under a 16 h photoperiod using cool white fluorescent lights (50-60 µE/m2/s light intensity). Then, were incubated under complete darkness at 25°C as control in an incubator with heat treatments of 35 and 45°C for 90 days. Microtubers started to develop at the terminal or axillary ends of the shoots within 20-25 days and they were ready for harvesting after 80-90 days, depending upon genotype. The harvested parameters were microtubers number/ propagule, microtubers weight/ plant (g), average microtubers length (cm) and average microtubers diameter (cm). The harvested microtubers were cold stored and used as minitubers seed for greenhouse culturing.

3. Biochemical Markers Analyses

3.1. Total glutathione content

The level of glutathione in potato shoots was determined according to Moron et al. (1979), includes combining GSH with DTNB (5,5'-dithiobis nitro benzoic acid) to produce a yellow product that absorbs at 412 nm.

3.2. Lipid peroxidation content

Reactive oxygen species degrade lipids, making them one of the most sensitive cellular components (by peroxidation of unsaturated fatty acids in biological membranes). The TBARS (Thiobarbituric Acid Reactive Substances) assay is a well-known method for determining lipid peroxidation. The level of lipid peroxidation in potato shoots samples was measured in terms of estimating the end product, MDA (malondialdehyde) (Heath and Packer, 1968) with some modifications. About 0.5 g from potato shoots was homogenized in 2.5 mL of 0.1% (w/v) trichloroacetic acid (TCA). The homogenate was centrifuged at 14.000 rpm for 15 min. 5 % thiobarbituric acid (TBA, 2.0 mL) was mixed with 20% TCA solution and the mixture was added to 0.5 mL of the liquid supernatant of potato samples. The mixture was heated at 95 ° C for 30 mins in a water bath and then incubated in an ice bath for 5 min. After centrifugation, the supernatant was read at 532 nm. The value for nonspecific turbidity of each sample at 600 nm was also recorded and subtracted from the absorbance recorded at

532 nm. The concentration of MDA-TBA adduct was calculated from MDA standard curve and converted to nmol g^{-1} fresh weight.

4. Nanoparticles Applications

Pure preparation of SiO_2 nano powder (99%); 25 to 55 nm in size, was purchased from Sigma-Aldrich (Germany). The NPs were sonicated in sterilized MS medium to final concentrations of 0.5 or 2 mg L^{-1}. Plantlet fresh weight (CFW) was recorded after 30 and 45 days of incubation.

5. Statistical Analysis

Data of experiments were statistically analyzed as completely randomized design with four replicates. The recorded data were analyzed statistically using analysis of variance technique (ANOVA) and by multiple range tests (Steel et al., 1997). Duncan's Multiple Range Test as described by Duncan (1955) and Least Significant Difference (LSD) at $p \leq 0.05$ level of confidence was employed to compare the differences among means.

RESULTS AND DISCUSSION

1. *In Vitro* Propagation and Microtuberization Under Heat Stress Conditions

For studying the effectiveness of SiO_2 NPs in elevating the negative effect of heat stress on micropropagation, microtubers formation and some biochemical characters, two experiments have been conducted *in vitro* using virus-free-mericlones stock plants of four potato cultivars (Agria, Cara, Diamond, and Hermes).

1.1. Vegetative and harvesting parameters

1.1.1. Agria cultivar

Results in table (1) and fig. (1) show the effect of SiO_2 NPs treatments under different levels of heat stress on vegetative characteristics of Agria potato cultivar after 4 weeks.

Regarding the effect of different treatments of SiO_2 NPs under heat stress levels on shoot length and number of shoots/explant in Agria cultivar, the results in table (1) indicate that 10 ppm SiO_2NPs at 35°C recorded the significantly highest shoot length (14.67 cm). The same treatment recorded the highest number of shoots (7) without significant differences with treatments of 5 and 10 ppm SiO_2NPs at 25 and 45°C. The highest root length (5 cm) was recorded with 10 ppm SiO_2 NPs at 35°C without significant differences with the same treatment of SiO_2-NPs at 25°C (4 cm), control treatment at 35 and 45°C and 20 ppm SiO_2-NPs at 45°C. The highest number of roots was obtained with 10 ppm SiO_2 NPs at 35°C (10.33) and 25°C (9.67) without significant differences between them. Meanwhile, control treatment without SiO_2 NPs under 25°C SiO_2 NPs at 5 ppm under 35°C and 20 ppm SiO_2 NPs under 25°C were not able to form any roots.

Table (1). Effect of SiO_2-NPs under different levels of heat stress on shoot length, shoot number, root length and root number of Agria potato cultivar after four weeks.

SiO2-NPs conc. (ppm) / Temperature (°C)	0	5	10	20	0	5	10	20
	Shoot length (cm)				Shoot number/ explant			
25	7.67cde	5.33f	8.33bc	8.00bcd	3.33c	6.00ab	6.00ab	4.67bc
35	7.00cdef	5.67ef	14.67a	6.00def	3.00c	3.67c	7.00a	3.67c
45	5.33f	5.00f	10.00b	8.00bdc	3.67c	5.00abc	6.00ab	4.33bc
	Root length (cm)				Root number/ explant			
25	0.00e	0.73de	4.00ab	0.00e	0.00d	3.00bc	9.67a	0.00d
35	4.67ab	0.00e	5.00a	1.67cde	5.00b	0.00d	10.33a	4.33b
45	3.17abc	2.67bcd	0.93de	3.50abc	2.77bc	2.00bc	1.00c	1.00c

Results in table (2) and fig. (1) show the effect of SiO_2 NPs under different levels of heat stress on the harvested Agria microtuber characteristics *in vitro* under complete darkness after 90 days.

Table (2). Effect of SiO_2 NPs under different levels of heat stress on microtuber weight/plant, number, length and diameter of Agria potato cultivar after three months under complete darkness.

SiO2-NPs conc. (ppm) / Temperature (°C)	0	5	10	20	0	5	10	20
	Microtubers weight/plant (g)				Microtubers number			
25	0.37bc	0.49b	0.26bcd	0.40bc	2.67bc	5.33a	4.00ab	3.67ab
35	0.00d	0.52b	0.43b	0.29bc	0.00c	2.00bc	3.33ab	1.67bc
45	0.13cd	0.89a	0.79a	0.93a	1.33bc	2.00bc	1.33bc	2.00bc
	Microtubers length (cm)				Microtubers diameter (cm)			
25	0.47b	0.53b	0.53b	0.53b	0.40a	0.30a	0.33a	0.37a
35	0.00c	0.30bc	0.60b	0.33bc	0.00b	0.80a	0.40a	0.77a
45	0.47b	1.43a	1.43a	1.10a	0.27a	0.70a	0.80a	0.70a

Referring to results in table (2), they show that, the highest value of microtubers weight/plant were recorded at 45°C without significantly differences between treatments of SiO_2-NPs of 5, 10 and 20 ppm (0.89, 0.79 and 0.93 g, respectively). For number of microtubers, 5 ppm of SiO_2 NPs at 25°C appeared to be the significantly highest number of microtubers (5.33), whereas the control treatment at 35°C cannot able to give any microtubers. As for microtubers length, the highest length of 1.43 cm was recorded with 5 and 10 ppm of SiO_2 NPs at 45°C. Nevertheless, SiO_2 NPs treatments had no significant effect on microtubers diameter with all heat stress levels.

1.1.2. Cara cultivar

Results in table (3) and fig. (2) show the effect of SiO_2 NPs under different levels of heat on the vegetative characteristics of Cara potato cultivar *in vitro* after 4 weeks.

Table (3). Effect of SiO_2 NPs under different levels of heat stress on shoot and root length, and number of Cara potato cultivar after four weeks.

SiO₂-NPs conc. (ppm) / Temperature (°C)	0	5	10	20	0	5	10	20
	Shoot length (cm)				**Shoot number/ explant**			
25	5.00c	10.00ab	5.00c	10.67a	3.00bcd	3.33abc	3.00bcd	3.33abc
35	6.00c	9.33ab	6.33c	8.83b	3.00bcd	4.00ab	3.67abc	4.67a
45	5.33c	5.50c	4.67c	4.67c	2.33de	4.00ab	3.33abc	1.67e
	Root length (cm)				**Root number/ explant**			
25	3.00b	5.00b	3.00b	4.00b	5.00ab	4.33ab	4.33ab	4.33ab
35	3.00b	7.00a	5.00b	3.67b	1.67cd	3.00bc	6.00a	6.00a
45	0.17c	0.43c	0.13c	0.00d	2.33bcd	1.33cd	2.33bcd	0.00d

Concerning Cara shoots length, data in table (3) shows that the highest value (10.67 cm) was obtained when 20 ppm SiO_2 NPs was used at 25°C, followed by 5 ppm of SiO_2 NPs at 25°C and 35°C (10.0 and 9.33 cm), consecutively without significant differences among them. The highest shoot number (4.67) was also achieved on the same treatment of SiO_2 NPs (20 ppm) at 35°C, followed by 5 ppm of SiO_2 NPs at 35°C and 45°C (4.0), respectively without significant differences among them. Concerning root length, results cleared that 5 ppm SiO_2 NPs at 35°C recorded the significantly the highest value (7.0 cm). While the highest root number (6.0) were obtained with both 10 and 20 ppm SiO_2 NPs at 35°C, followed by all treatments of SiO_2 NPs at 25°C (4.33). While, treatment of 20 ppm of SiO_2 NPs at 45°C could not able to form any roots.

Results in table (4) and fig. (2) show the effect of SiO_2 NPs under different levels of heat stress on the characteristics of harvested Cara potato cultivar *in vitro* under complete darkness for 90 days.

Regarding the microtubers weight/ plant, the highest mean value (0.42 g) was recorded at 25°C without SiO_2 NPs, followed by 20 ppm of SiO_2 NPs at the same heat level (0.27 g) without a significant difference between them. About microtubers number, there were no significant differences among all the treatments, except 45°C with different SiO_2 NPs concentration, which did not form any microtubers. With reference to microtubers length, the highest mean value (0.30 cm) was recorded with control (without SiO_2 NPs) at 25°C heat level. Concerning microtubers diameter, the highest mean values were recorded without SiO_2 NPs at 25 and

45°C, 10 ppm of SiO_2 NPs at 35°C and 20 ppm of SiO_2 NPs at 25°C (0.23, 0.26, 0.27 and 0.27 cm, respectively) without significant differences among them.

Table (4). Effect of SiO_2 NPs under different levels of heat stress on microtubers weight, number, length and diameter of Cara potato cultivar after three months *in vitro* under complete darkness.

SiO2-NPs conc. (ppm) / Temperature (°C)	0	5	10	20	0	5	10	20
	Microtubers weight/plant (g)				**Microtubers number**			
25	0.42a	0.13bc	0.00d	0.27ab	1.00a	1.00a	0.00b	0.67a
35	0.06c	0.047c	0.08c	0.16bc	1.33a	0.67a	1.67a	1.33a
45	0.11bc	0.00d	0.00d	0.00d	1.67a	0.00b	0.00b	0.00b
	Microtubers length (cm)				**Microtubers diameter (cm)**			
25	0.30a	0.13ab	0.00b	0.23ab	0.23a	0.07ab	0.00b	0.27a
35	0.10ab	0.10ab	0.20ab	0.17ab	0.10ab	0.07ab	0.27a	0.10ab
45	0.23ab	0.00b	0.00b	0.00b	0.26a	0.00b	0.00b	0.00b

1.1.3. Diamond cultivar

Results in table (5) and fig. (3) show the effect of SiO_2 NPs under different levels of heat stress on vegetative characteristics of Diamond potato cultivar after four weeks.

Regarding the vegetative characteristics, table (5) shows that the maximum mean shoot length (15 cm) was achieved with both 10 and 20 ppm SiO_2 NPs at 25°C and 20 ppm SiO_2 NPs at 45°C without significant differences with the control treatment at 25°C (13.67 cm). About shoot number, the highest shoot number/ explant (5.0) was recorded with 5ppm of SiO_2 NPs at 35°C, 10 ppm of SiO_2 NPs at 25°C and 20 ppm of SiO_2 NPs at 25°C and 45°C. While, 10 ppm of SiO_2 NPs at 45°C gave the lowest shoot number among all treatments (0.67). The highest mean root length was ranged between 6.67 cm and 4.67 cm with control treatment at 35°C and with treatments of SiO_2 NPs (5, 10 and 20 ppm) at 25°C and 35 °C with significant differences among the rest of the treatments. Referring to root number, 5 ppm SiO_2 NPs at 25°C was recorded the highest mean value (5.67), followed by 10 and 20 ppm SiO_2 NPs at same heat level and the treatment with 20 ppm SiO_2 NPs at 45°C without significant differences among them.

Results in table (6) and fig. (3) show the effect of SiO_2 NPs under different levels of heat stress on microtubers of the characterization of harvested Diamond potato cultivar *in vitro* under complete darkness after 90 days.

Table (5). Effect of SiO_2 NPs under different levels of heat stress on shoot and root length shoot number /explant of Diamond potato cultivar after four weeks.

SiO2 NPs conc. (ppm) / Temperature (°C)	0	5	10	20	0	5	10	20
	Shoot length (cm)				Shoot number/ explant			
25	13.67a	9.33bcd	15.00a	15.00a	3.67abc	4.33ab	5.00a	5.00a
35	10.00bc	11.33b	8.00cd	7.00de	3.00bcd	5.00a	3.67abc	2.00cde
45	5.00e	7.33de	7.00de	15.00a	2.00cde	1.33de	0.67e	5.00a
	Root length (cm)				Root number/ explant			
25	3.00b	5.17a	6.67a	5.33a	3.67ab	5.67a	4.00ab	4.00ab
35	5.00a	5.00a	6.33a	4.67ab	3.33abc	3.33abc	3.00bc	3.33abc
45	0.33c	0.33c	0.33c	0.33c	0.67d	0.55d	1.00cd	4.00ab

Table (6). Effect of SiO_2 NPs under different levels of heat stress on microtubers weight, number, length and diameter of Diamond potato cultivar after three months *in vitro* under complete darkness.

SiO2-NPs conc. (ppm) / Temperature (°C)	0	5	10	20	0	5	10	20
	Microtubers weight/plant (g)				Microtubers number			
25	0.11a	0.50a	0.00b	0.29a	0.67a	1.33a	0.00b	1.00a
35	0.00b	0.27a	0.43a	0.37a	0.00b	1.00a	0.67a	1.33a
45	0.00b	0.23a	0.00b	0.29a	0.00b	1.00a	0.00b	1.00a
	Microtubers length (cm)				Microtubers diameter (cm)			
25	0.10a	0.50a	0.00b	0.33a	0.07a	0.27a	0.00b	0.20a
35	0.00b	0.27a	0.27a	0.43a	0.00b	0.23a	0.23a	0.33a
45	0.00b	0.30a	0.00b	0.33a	0.00b	0.17a	0.00b	0.20a

Concerning the harvested characteristics, represented in table (6), there were no significant differences of the treatments of SiO_2 NPs under different levels of heat stress with a low mean value for all parameters (microtubers weight, number, length and diameter), and absence of microtubers formation was observed in the control treatment without SiO_2 NPs under 35°C and 45°C and the treatment of 10 SiO_2 NPs under 25°C and 45°C.

1.1.4. Hermes cultivar

Results in table (7) and fig. (4) show the effect of SiO_2 NPs under different levels of heat on vegetative characteristics of Hermes potato cultivar after four weeks *in vitro*.

Table (7). Effect of SiO_2 NPs under different levels of heat stress on shoot and root length of Hermes potato cultivar after four weeks.

SiO_2-NPs conc. (ppm) / Temperature (°C)	0	5	10	20	0	5	10	20
	Shoot length (cm)				**Shoot number/ explant**			
25	5.00bc	12.33bc	10.00bc	23.00a	3.00bc	4.33ab	4.33ab	6.00a
35	13.67b	9.00bc	8.00bc	10.00bc	4.33ab	2.67bc	2.33bc	3.00bc
45	5.00bc	4.33c	5.00bc	7.00bc	2.00c	3.00bc	3.00bc	3.00bc
	Root length (cm)				**Root number/ explant**			
25	3.00bc	0.00e	2.33c	5.00a	5.00d	0.00g	11.67b	15.33a
35	5.00a	3.00bc	4.00ab	5.00a	8.33c	4.67d	4.33de	3.00def
45	0.33d	4.67a	0.67d	0.50d	8.33c	4.67d	4.33de	3.00def

Concerning the shoot parameters, as shown in table (7), the significantly highest length of shoots (23.0 cm) was recorded when 20 ppm SiO_2 NPs was used at 25°C comparing to other treatments. Also, the same treatment of 20 ppm SiO_2 NPs gave the highest shoot number (6.0), followed by the control treatment (without SiO_2 NPs) at 35°C, 5 and 10 ppm SiO_2 NPs at 25°C (4.33) without significant differences among them. However, root length recorded the highest mean value (5.00 cm) with 20 ppm SiO_2 NPs at 25 and 35°C and the control (0.00 ppm SiO_2 NPs) at 35°C. The root length recorded 4.67 cm with 5 ppm SiO_2 NPs at 45°C, followed by root length of 4.0 cm when 10 ppm SiO_2 NPs was used at 35°C without significant differences among them. Whereas the significantly highest root number (15.33) was recorded with 20 ppm SiO_2 NPs at 25°C. This treatment followed by 10 ppm SiO_2 NPs at same heat level (11.67) with significant differences between them. While, the treatment of 5 ppm SiO_2 NPs at 25°C did not from any roots.

Results in table (8) and fig. (4) show the effect of SiO_2 NPs under different levels of heat stress on the characteristics of harvested microtubers of Hermes potato cultivar *in vitro* under complete darkness after 90 days. Results in table (8) reveal that the highest microtubers weight/plant was 0.76 g with 5 ppm SiO_2 NPs at 25°C, followed by 0.60 g with 20 ppm SiO_2 NPs at the same temperature without significant differences between them. Concerning of microtubers number, 20 ppm SiO_2 NPs gave the highest mean value (2.67) at 25°C, followed by microtubers number of 2.00 with 5 and 10 ppm SiO_2 NPs at the same temperature (25°C) without significant differences among them. Meanwhile, treatments of 10and 20 ppm SiO_2 NPs at 35°C and all treatments at 45°C were not able to form any microtubers. About microtubers length, the significantly highest mean value was 1.13 cm when 5 ppm SiO_2 NPs was used at 25°C with significant differences among all treatments. Also, microtubers diameter was recorded the significantly highest mean value (0.50 cm) with the treatment of 20 ppm SiO_2 NPs at 25°C with significant differences among all treatment.

Table (8). Effect of SiO_2 NPs under different levels of heat stress on microtubers weight/plant, number, length and diameter of Hermes potato cultivar after three months *in vitro* under complete darkness.

SiO₂-NPs conc. (ppm) / Temperature (°C)	0	5	10	20	0	5	10	20
	Microtubers weight/plant (g)				**Microtubers number**			
25	0.40bc	0.76a	0.47bc	0.60ab	1.00bcd	2.00ab	2.00ab	2.67a
35	0.43bc	0.20cd	0.00e	0.00e	1.33bc	0.67d	0.00e	0.00e
45	0.00e	0.00e	0.00e	0.00e	0.00e	0.00e	0.00e	0.00e
	Microtubers length (cm)				**Microtubers diameter (cm)**			
25	0.30bc	1.13a	0.33bc	0.47b	0.23b	0.23b	0.23b	0.50a
35	0.40b	0.17bc	0.00d	0.00d	0.21b	0.10bc	0.00d	0.00d
45	0.00d	0.00d	0.00d	0.00d	0.00d	0.00d	0.00d	0.00d

Generally, potato is highly sensitive to high temperature (Levy and Veilleux, 2007), presenting an obstacle to cultivation in tropical and sub-tropical areas. Potato plants that are exposed to high temperature from the beginning of the growing period have a risk of a high reduction in tuber yield compared to those with later exposure to high temperature, due to a delay in tuber initiation and shorter bulking duration, as well as a lower net assimilation rate (Aien et al., 2016). Climate change has been predicted to decrease the global potato yield from 18% to 32% without adaptation or from 9% to 18% with adaptation (Hijmans, 2003). In this context, adaptation is considered in the narrow sense of the “autonomous” adaptations made to the farmer field, such as adjusting the planting time or using adaptive cultivars (Hijmans, 2003).

At 45°C, Agria cv. produced the highest value of microtubers weight and length when SiO_2 NPs were used, followed by Diamond cultivar. In the contrast, Mohamed et al. (2016) indicated that Diamond cultivar could mark as moderately tolerant, while the Agria was ranked among the sensitive genotypes to heat stress at 30°C compared to the control (12°C and 24°C) through *in vitro* screening under normal conditions.

In the other studies on heat stress, Soundararajan et al. (2014) treated *Salvia splendens* with Si under high temperature (35ºC), and found that the activities of superoxide dismutase (SOD), Ascorbate peroxidase (APX) and Glutathione peroxidase (GPX) were increased while catalase (CAT) was decreased. Shalaby et al. (2021) found that foliar application of Si (200 mg L^{-1}) was more effective in alleviating salinity and heat stress of cucumber, recording the highest fruit yields under net-house conditions compared to other treatments (nano-selinum and H_2O_2) and the control. Also, spraying the plant leaves by Si and Se together protect garlic plants from the high temperature stress during germination and growth initiations, which reflects on germination percentage, germination speed, vegetative growth and bulb characters as well as total green, marketable yield and its quality (El-Zohiri and Farag, 2015).

The results of the present study agree with Kafi et al. (2019), who concluded that silicon application may be an effective strategy in reducing salinity effects and more efficiency will be achieved by using this element as nanoparticles in potato. Also, Gowayed et al. (2017) cleared that the application of SiO_2 NPs at the appropriate dose rate significantly enhances growth traits in salt-stressed potato plants and potato plantlets adapted to salt stress to some extent through increasing the activities of antioxidant enzymes, such as Glutathione peroxidase (GPX) and superoxide dismutase (SOD) and recommend using SiO_2 NPs at 50 mg L^{-1} as an optimized dose to improve plant growth under salinity stress.

2. Biochemical Analysis Results

2.1. Glutathione content

In plant cells, glutathione is a tripeptide that interacts chemically with other free radicals. GSH preserves the membrane structure by preventing acyl peroxide-induced lipid peroxidation (maintains membrane integrity). It assists in the detoxification of xenobiotics as a substrate for glutathione-S-transferase enzyme and a precursor of phytochelatin as a substrate for glutathione-S-transferase enzyme and a precursor of phytochelatine that function as heavy metals that bind peptides in plants. GSH is the most common non-protein thiol molecule found in plants and animals (Foyer et al., 1994).

Table (9) shows the effect of SiO_2 NPs on potato shoots subjected to different regimes of temperature. Glutathione is determined in shoots for determining the resistance of plants to heat stress. In Agria cv., the highest mean record was detected by 20 ppm at 25°C, but GSH level declined at 35°C and 45°C. In Cara cv., the levels of SiO_2 NPs at 25 and 35°C caused a decline in the level of GSH in samples when compared to the control. In Diamond cv., an increase in GSH level was noticed with 20 ppm SiO_2 NPs at 25°C, but decreased at 35°C. In Hermes cv., at 25°C with 5 ppm SiO_2 NPs application, the highest value was recorded but at 35°C, GSH was declined. These results go in the same line with Hassan et al. (2018) on heat stressed wheat treated with NPs, Mahdi et al. (2020) on saline stressed wheat. A fluctuation in the level of GSH under the treatment with NPs due to the effect of NPs differs from plant to other, stage of application of NPs and dose of NPs treatment.

Table (9). Effect of SiO_2 NPs on glutathione tripeptide µmol/g fresh weight of four potato cultivars *in vitro* under different heat stress levels.

SiO2-NPs conc. (ppm) / Temperature (°C)	0	5	10	20	0	5	10	20
	Agria cv.				**Cara cv.**			
25	5.40	8.17	9.98	39.39	37.42	16.02	6.75	8.39
35	9.45	8.95	3.42	1.56	18.52	13.48	15.00	8.46
45	4.19	6.04	----	----	L	L	L	L
	Diamond cv.				**Hermes cv.**			
25	15.90	8.96	9.14	40.92	18.45	35.42	8.66	3.01
35	90.69	1.13	3.88	8.18	31.18	17.10	3.37	1.56
45	L	L	L	L	L	L	L	L

L: lethal, ---: no shoots.

1.2. Lipid peroxidation level

Data in table (10) clear that, in Agria cv., the lowest level of the toxic product MDA was detected with the control, but increased in other treatments at 25°C. However, at 35°C, the minimum level of MDA was recorded with 20 ppm SiO_2 NPs. The highest temperature of 45°C increased the accumulation of MDA. In Cara cv., the treatment of 5 ppm SiO_2 NPs recorded the lowest level of MDA in both 25°C and 35°C. In Diamond cv., at 25°C, the lowest record of MDA was determined with 20 ppm SiO_2 NPs application, but at 35°C, the level of MDA decreased in all other treatments. In Hermes cv., the level of MDA recorded slight increase in MDA at 25°C, compared to the control. However, at 35°C, 20 ppm SiO_2 NPs recorded the lowest level of MDA. Results are agreed with Fouda et al. (2021), on *Salvadora persica* and Hassan et al. (2018) on wheat. Heat stress increases level of peroxidation and releasing MDA, which caused releasing in reactive oxygen species (Mahdi, 2011).

Table (10). Effect of SiO_2 NPs on lipid peroxidation level (MDA) in nmol/gm fresh weight of four potato cultivars *in vitro* under different heat stress levels.

SiO2-NPs conc. (ppm) / Temperature (°C)	0	5	10	20	0	5	10	20
	Agria cv.				**Cara cv.**			
25	3.10	4.11	4.56	11.24	9.36	5.48	26.30	23.21
35	16.09	11.96	8.49	5.16	11.76	2.13	33.64	18.66
45	4.15	5.77	----	----	L	L	L	L
	Diamond cv.				**Hermes cv.**			
25	11.70	10.25	20.83	4.59	3.16	3.51	6.07	14.10
35	7.13	9.39	11.48	17.99	12.38	4.31	9.50	-0.46
45	L	L	L	L	L	L	L	L

L: lethal, ---: no shoots.

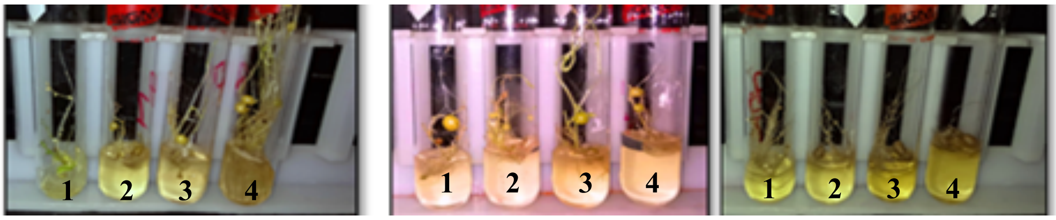

Fig. (1). The effect of SiO_2-NPs at (1) 0.00, (2) 5, (3) 10 and (3) 20 ppm under different levels of heat at (A) 25, (B) 35 and (C) 45C° on vegetative and harvested parameters, of “Agria" cv. *in vitro*.

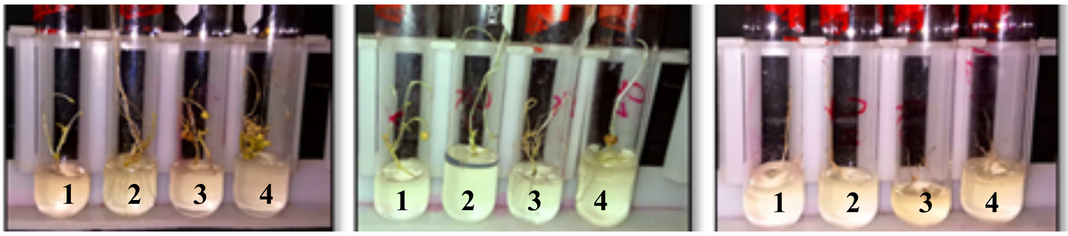

Fig. (2). The effect of SiO2-NPs at (1) 0.00, (2) 5, (3) 10 and (3) 20 ppm under different levels of heat at (A) 25, (B) 35 and (C)45C°on vegetative and harvested parameters, of “Cara" cv. *in vitro*.

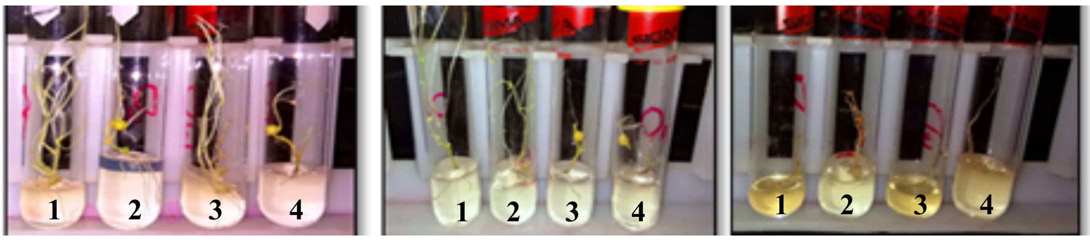

Fig. (3). The effect of SiO_2-NPs at (1) 0.00, (2) 5, (3) 10 and (3) 20 ppm under different levels of heat at (A) 25, (B) 35 and (C) 45C°on vegetative and harvested parameters, of “Dimond" cv. *in vitro*.

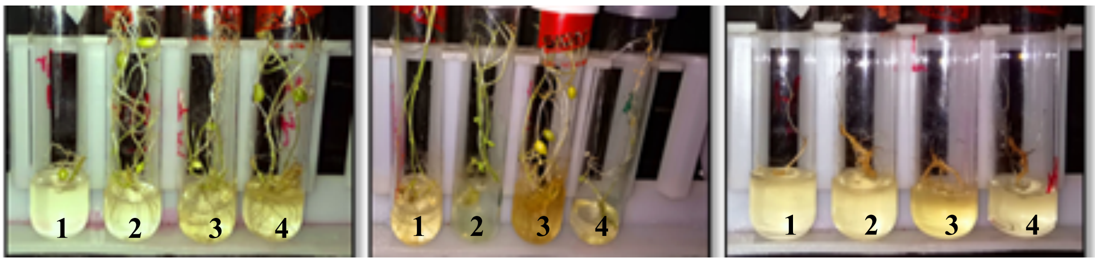

Fig. (4). The effect of SiO_2-NPs at (1) 0.00, (2) 5, (3) 10 and (3) 20 ppm under different levels of heat at (A) 25, (B) 35 and (C) 45C°on vegetative and harvested parameters, of “Hermes" cv. *in vitro*.

CONCLUSIONS

This is the first study on the effect of SiO_2 NPs on microtuberization of potato under heat stress condition. The obtained results show that all microtubers parameters in Agria cultivar are positively and significantly affected by SiO_2 NPs treatments followed by Hermes, and Cara. On the other hand, there was no significant effect of SiO_2 NPs on Diamond cv, under different heat levels. At 45°C, Agria cv. produced the highest value of microtubers weight and length, followed by diamond. While both Herms and Cara did not form any microtubers at the same heat level. According to the results, the four potato cultivars were ranked for heat tolerance based on vegetative, harvested and biochemical parameters with SiO_2 NPs. Agria ranked first, followed by Diamond, then Hermes, and the last cultivar was Cara. The agri-nanotechnology is considered one of the most important and promising issues in this context. It is found that, nanomaterials can alleviate the damage resulting from different abiotic stresses through activating process of plant defense system. Nanomaterial also could improve the productivity, positive morphological effects like improvement germination rate and percentage; shoot and root length.

This finding is very important for the commercial production of potato under combined stresses in arid and semi-arid countries. The biotechnology techniques as agricultural practices are promising tools in ameliorating the adverse impact of heat stress particularly for the potato production during summer months from May to August in arid and semi-arid regions. In future investigations, combination of nanoparticle elements should be performed to evaluate the synergism of these beneficial elements to increase tolerance of potato plants to drought, salinity and heat stress.

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تحسين القدرة على تحمل الحرارة في البطاطس

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تعتبر درجة الحرارة أحد الضغوط غير الحيوية الرئيسية التي تؤثر على نمو البطاطس وإنتاجيتها في المناطق شبه القاحلة ومناطق النمو. يمكن أن يؤدي استخدام مناهج التكنولوجيا الحيوية الجديدة وتطبيق المواد النانوية إلى تحسين إنتاجية وجودة البطاطس. أثبت استخدام بعض العناصر في صورة النانو مثال نانو السيليكون (SiO_2 NPs) إلى تعزيز النمو والإنتاجية في ظل الضغوط المختلفة مثل الملوحة والجفاف والمعادن الثقيلة. في هذه الدراسة، تم اختبار فاعلية النانو سيلكون عند ٥، ١٠ و٢٠ جزء في المليون، تحت مستويات مختلفة من الحرارة عند ٢٥، ٣٥ و٤٥ درجة مئوية. ودراسة تأثيرها على النمو والقياسات البيوكيميائية لأربعة أصناف من البطاطس في المعمل. أشارت النتائج إلى تفوق الصنف أجريا والذي أعطى تأثير إيجابي معنوي مع تركيزات السيليكون، يليه الصنف هيرمس وكارا. من ناحية أخرى لم يكن هناك تأثير معنوي لـلسيليكون على الصنف دايمونت تحت مستويات الحرارة المختلفة. عند ٤٥ درجة مئوية، أنتج الصنف أجريا أعلى قيمة لوزن وطول الدرنات الدقيقة، يليه الصنف دايمونت مع التركيزات ٥ و٢٠ جزء في المليون من النانو سيلكون. كانت أعلى قيم للصفات الخضرية لصنف أجريا عند استخدام ١٠ جزء في المليون من النانو سيلكون عند ٣٥ درجة مئوية. بالنسبة لصفات الحصاد لنفس الصنف كانت أعلى قيمة لوزن الدرنات الدقيقة عند ٤٥ درجة مئوية دون تأثير معنوي بين تركيزات السيليكون في الصورة النانو. أعلى طول للدرنات الدقيقة كان عند ٥ و١٠ جزء في المليون من تركيزات السيلكون عند حرارة ٤٥ درجة مئوية. وبالنسبة لقطر الدرينة فإنه لا يوجد أي تأثير معنوي لتركيزات السيلكون تحت كل مستويات الحرارة. أيضًا، كانت قيمة الجلوتاثيون GSH الأعلى بمقدار ٥ جزء في المليون عند ٤٥ درجة مئوية. فيما يتعلق بمستوى المالون داي الدهايد MDA، فإن أدنى مستوى من المادة السامة MDA كان في عدم وجود النانو سيليكون، وزاد في المعاملات الأخرى عند درجة حرارة ٢٥ مئوية وعند ٣٥ درجة مئوية. تم تسجيل الحد الأدنى من MDA عند التركيز ٢٠ جزء في المليون. وبشكل عام، أدت أعلى درجة حرارة (٤٥ درجة مئوية) إلى زيادة تراكم MDA.