Molecular Characters of Potato Explants as Affected by Silicon Nanoparticles under Drought Stress

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Abstract: Grades of microtubers of four potato cultivars (Hermes, Charlotte, Inova and Maris Peer) under two concentrations of Si-NPs (200 and 400 ppm) exposed to drought stress, induced by polyethylene glycol (PEG 10%) were evaluated. Si-NPs at low concentration-treated explants gave the maximum number and weight (7 and 0.89 g, respectively) of grade 1 of microtubers in Hermes cv. Shoots and microtubers of Hermes cv. showed presence or absence of specific isoenzymes of peroxidase (POD), polyphenol oxidase (PPO) and amplification of specific amplicons with different base pairs using 5 different SCoT primers. Isoenzyme of POD1 was present in microtubers and both isoenzyme of PPO 8 in microtubers and PPO10 isoenzyme in shoot were absent. The highest number of total monomorphic and polymorphic bands (23) in microtubers amplified with different 5 SCoT primers were recorded. Absence of amplicons with 700 and 460 bp amplified using SCoT 1 and 510bp with SCoT11 and presence of both amplicons 645 and 280 bp with SCoT11in microtubers were also detected. The same concentration of Si-NPs gave the maximum number and weight (7 and 0.17 g, respectively) of grade 2 of microtubers in Inova cv. Isoenzyme of POD10 was absent in shoot and isoenzymes of PPO 8, 9 in shoot and isoenzyme of PPO8 in microtubers were absent. Amplicons with 375 bp with SCoT 8 in shoots was disappear. It can recommend that, application of 200 ppm of Si-NPs was more effective for large grade microtubers induction *in vitro* especially in Hermes and Inova cvs.

Keywords: *Solanum tuberosum* L., microtubers grades, in vitro, Nanoparticles, drought stress, genetic polymorphism

INTRODUCTION

Potato (*Solanum tuberosum* L.) is the fourth most important crop worldwide for consumption and exportation. Tuber is a differentiated stem that developed from the underground stolon. Potato is an essential source of low-fat source of carbohydrates, antioxidants, protein, vitamins, macro and micronutrients, polyphenols, carotenoids, and tocopherols (Brown, 2005). To maintain a sustainable potato production under different abiotic stressors as drought, it must adapt the cultivation practices and develop stress tolerant potato cultivars that are appropriately engineered for changing environment. *In vitro* selection methods using polyethylene glycol (PEG), identify drought-potato tolerant strains, which might also be *ex vitro* drought tolerant. Understanding the physiological, biochemical, and molecular mechanisms of tuber induction and formation especially under drought, is of great significance for potato cultivations and production. Drought during tuberization, causes reduction of stolon number per stem, which lower tuber number and yield. Stolon initiation and tuber formation are the most critical stages of potato under drought stress (Dahal *et al.,* 2019).

Drought or drought stress had negative effects on growth and development of plants due to regeneration of different kinds of Reactive oxygen species (ROS). ROS as superoxide radicals (O_2), hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$), and hydroxyl radicals (OH), interacted with cellular macromolecules as DNA, proteins, and lipids. Plants had highly effective complex enzymatic antioxidant system as superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), polyphenol oxidase (PPO), ascorbate peroxidase (APX), guaiacol peroxidase (GPX), glutathione reductase (GR) and non-enzymatic antioxidants as, ascorbate, phenolic compounds, glutathione etc., can scavenge these harmful ROS (Zhu, 2002). PPO is a nuclear encoded enzyme that catalyzes the oxygen-dependent oxidation of phenols to quinones. However, peroxidase play an important role in cell wall biosynthesis, response to injury, disease resistance, and wound repair, oxidation of indole-3-acetic acid, hydoxylation of proline as well as salt and drought tolerance of potato (Rahnama and Ebrahimzadeh, 2005). PPO levels in a plant increase when a plant is wounded or infected. It extracted and purified from potato tubers and found that it has molecular weight 86 KDa, composed of two identical subunits and 2 Cu^{2+} ions per enzyme molecule. Polyphenol oxidase enzymes oxidated the phenolic compounds to quinones, formed brown pigments known as melanin, which cause a major losses during the processing into flakes, chips, and frozen French fries (Yong and Ahn, 2007). However, Elhamahmy *et al.* (2021) observed that, gamma-treated plantlets of potato cultivars, Lady Rosetta, Diamante, and Gold showed higher activity of peroxidase (POD) and polyphenol oxidase (PPO) under salt stress. Isoenzyme's analysis showed an absence of POD number 3, 4, and 5 in Gold cv. plantlets (susceptible to salinity). The dye of most POD and PPO bands were denser (more active) in gamma-treated plantlets of Santana cv. as compared to other cultivars. Both gamma-treated and untreated plantlets showed the absence of PPO number 1 in Lady Rosetta and Diamante cvs. and PPO number 3, 4, and 5 in Gold cv. plantlets.

Start Codon Targeted (SCoT) polymorphisms are reproducible markers that are constructed on the short-conserved region in plant genes close the ATG

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translation start codon. Using of SCoT markers was more efficient tool compared to other arbitrary markers, because of the longer primer distances and high annealing temperatures (Collard and Mackill, 2009). The SCoT marker design method does not need fully genomic sequence information, making it easier to apply to plants that do not have a reference genome as potato (Xiong *et al*., 2009). SCoT analysis was more effective in fingerprinting of potato varieties than other techniques as inter simple sequence repeat (ISSR) and random amplified polymorphic DNA (RAPD) (Gorji *et al*., 2011). Gupta *et al*. (2019) showed that, SCoT marker had many advantages over the other commonly used molecular markers, so it was more stability and producing dependable bands. Also, SCoT marker was easy to design, highly polymorphic and provided ample genetic information with *Capsicum* accessions. Abd El-Moneim *et al*. (2021) found that, SCoT analysis revealed high polymorphism percentages (85.26%) among five different quinoa (*Chenopodium quinoa* Willd) genotypes at different salinity levels. SCoT 7 attained the greatest number of polymorphic amplicons (26). Khatab *et al.* (2021) reported that six SCoT primers generated high polymorphic percentage with 43 polymorphic bands for wheat genotypes tolerate to salinity. SCoT 16 with sequence ACCATGGCTACCACCGAC, gave 90% of polymorphic % among cultivars.

Therefore, this research aimed to study the effect of Si-NPs at concentrations (200 and 400 ppm) on microtuber grades of four potato cultivars (Hermes, Charlotte, Inova and Maris Peer) under drought stress induced by polyethylene glycol (PEG 10%). Also, potato explants and microtubers were investigated under molecular (peroxidase and polyphenol oxidase isoenzymes, amplification of specific genetic fragments (amplicons) using five different primers (SCoT 1, 3, 6, 8 and 11) to determine the genetic polymorphism among cultivars and treatments.

MATERIALS AND METHODS

In Vitro Culture of Potato Cultivars and Drought Stress Induction:

Tubers of four potato cultivars (Hermes, Charlotte, Inova and Maris Peer) were sterilized with Clorox 10% for 3 min. and 70% of ethanol for 30 sec. Then soaked in 50 ppm of GA_3 to break dormancy (Momena *et al.,* 2014). Bud sprouts were cultivated after sterilization with $HgCl_2$ (0.1%) on hormone-free MS medium with 30 g/ L of sucrose, 7 g/ L of agar for 70 d (Murashige and Skoog, 1962). Explants were exposed to Polyethylene glycol (PEG-6000, Sigma) at 10% to initiate osmotic potential -1.48 MPa, as described by (Michel and Kaufmann, 1973). Sucrose at 8% and 4 ppm of BAP were added. Si-Nanoparticles (Sigma) with 100 nm size, at 200 and 400 ppm were applied. Explants were incubated in the dark conditions for 90 d at 18-20°C, 60-70% of humidity. Each treatment was replicated 10 times and each Jar contained 5 explants. After 160 days from subculture, explants were harvested. Some vegetative and molecular characters were estimated as follow:

Microtubers Grades:

Number and weight (g) of microtubers of Grade 1 (Diameter > 4 mm), Grade 2 (Diameter 2: 4 mm), Grade 3 (Diameter < 2 mm) were also investigated.

Isoenzymes of POD and PPO:

Polyacrylamide gel electrophoresis (PAGE) was performed to identify isoenzyme variations, among the studied NPs shoots and microtubers as well as the control, using peroxidase (POD) and polyphenol oxidase (PPO) according to Mohammadi and Prasanna (2003). Isoenzymes were extracted by homogenizing half g of fresh samples in 1 ml extraction buffer (10% glycerol), and the extract was centrifuged at 10000 rpm for 5 minutes. The supernatant was used for electrophoretic analysis. A sample of 40 µl extract was mixed with 20 µl sucrose and 10 µ bromophenol blue, and then a volume of 50 µl of this mixture was applied to each well. Gel run was performed at 150 V until the bromophenol blue dye has reached the separating gel, and then voltage was increased to 200 V. Electrophoresis apparatus was placed inside a refrigerator during the run.

POD Staining and Detection:

After electrophoresis, the gel was stained with benzidine di-HCl (0.125 gm), glacial acetic acid (2 ml) and the mixture was toped up to 50 ml with distilled water. Gel was placed into this solution, and 5 drops of hydrogen peroxide was added. The gel was incubated at room temperature until bands appear as described by Yeh *et al.* (1999).

PPO Staining and Detection:

The gel was stained with 0.1 M of phosphate buffer (pH 6.5), 100 mg of sulfanilic acid, 200 mg catechol in 2 ml acetone. The gel was then placed into this solution and incubated at 30ºC for 30 min until the bands appeared, and then gels were scanned and analyzed using Gel Doc Vilber Lourmat system (Yong and Ahn, 2007).

SCoT Analysis:

Genomic DNA Extraction:

Genomic DNA of shoots and microtubers was extracted using 500 mg of samples with 400 µl extraction buffer containing 200 mM Tris–HCl (pH 7.5), 250 mM NaCl, 25 mM EDTA (pH 8.0) and SDS (0.5 %). This mixture was vortexed in Eppendorf tubes for 20 s, and warmed in water bath at 65°C for 15 min, and 200 µl sodium acetate (3 M) was added to the solution and centrifuged at 13.000 rpm for 10 min. The supernatant (500 µl) was transferred to new Eppendorf with 500 µl cold isopropanol, and shaken for 5 min. then centrifuged at 13.000 rpm for 10 min. The residual was washed with 200 µl cold ethyl alcohol (70%) then centrifuged at 13.000 rpm for 5 min. This step was repeated twice, and then 50 µl d H_2O was added to the dry residual and stored at 20°C overnight, followed by the addition of 1 µl RNAase (10 mg/1 ml H_2O) and incubation at 37°C for 1 h. This mixture was then provided with 150 µl dH_2O and 200 µl phenol and shaken well for 2 min., and then centrifuged at 13,000 rpm for 10 min. The supernatant

was mixed with 100 µl phenol, 100 µl chloroform and shaken for 2 min, followed by centrifugation at 13.000 rpm for 10 min. Dried residual was mixed with 50 µl d H_2O and stored at 20°C (Wulff *et al*., 2002). The primers names and their nucleotide sequences used for SCoT procedure are listed in Table (2). The primers SCoT 1 and 3 had similar sequence except the last four nucleotides 5′ ACGC 3′ in SCoT 1 and 5′ CACA 3′ in SCoT 3. Also, primers SCoT 8 and 11 had similar sequence except the last three nucleotides 5′ GAG 3′ in SCoT 8 and 5′ ACC 3′ in SCoT 11. The primer SCoT 6 was completely differed from other primers. All SCoT primers had ATG sequence.

Table (1): List of the primer names and their nucleotide sequences used for SCoT procedure

No.	primer Name	Sequence	Annealing temperature (°C)
1	**SCoT 1**	**5′** ACGACATGGCGACCACGC **3′**	**60**
2	**SCoT 3**	**5′** ACGACATGGCGACCCACA **3′**	**58**
3	**SCoT 6**	**5′** CAATGGCTACCACTACAG **3′**	**54**
4	**SCoT 8**	**5′** ACAATGGCTACCACTGAG **3′**	**54**
5	**SCoT 11**	**5′** ACAATGGCTACCACTACC **3′**	**54**

Polymerase Chain Reaction (PCR):

The amplification of DNA was performed in an automated thermal cycle (model Techno 512) programmed for one cycle at 94ºC for 4 min, followed by 45 cycles of 1 min at 94ºC, 1 min at 54-60ºC (annealing temperature differed according to primer sequence as shown in Table 2), and 2 min at 72ºC. The reaction was finally stored at 72ºC for 10 min. PCR was performed in 30 µl-volume tubes that contained 3 µl dNTPs (2.5 mM), 3 µl $MgCl_2$ (25 mM), 3 µl Buffer (10 x), 2 µl primer (10 pmol), 0.2 µl Taq DNA polymerase (5U/µl), 2 µl Template DNA (25 ng) and 16.8 µl H_2O (dw), according to Excof er *et al.* (1992). Agarose (1.5%) was warmed with 100 ml of TBE (Tris/ borate/EDTA) buffer, and then 5 µl ethidium bromide was added after the temperature became 55°C. Samples of DNA amplified product (15 µl) was loaded in each well. DNA ladder was used as standard DNA with molecular weights of 3000, 1500, 1000, 500 and 100 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).

RESULTS AND DISCUSSION

Effect of Si-NPs on Microtubers Grade Parameters:

Table (2) and Fig. (1) showed that, Si-NPs at low concentration-treated explants gave the maximum number and weight of grade 1 and 2 (7 and 0.89 g, in Hermes cv., respectively) and (7 and 0.17 g in Inova cv., respectively). PEG-stressed explants of Inova cv. recorded the highest number and weight of grade 3 (11 and 0.12 g, respectively). Results were agreed with Bent (2014) who found that 30 ppm of Si increased the potato yield by 6.2 % and improved the proportion of large grade (size) tubers. Also, Soratto *et al.* (2012), showed increment of potato tuber weight from 0.1 to 39.6 % according to variety as well as enhancement of the tuber dry biomass.

Table (2): Effect of Si-NPs on number and its weight of different grade of microtubers of potato cultivars under drought stress after 160 d from subculture

Cultivars	Treatments	Conc. (ppm)	Grade 1 (Diameter > 4 mm)		Grade 2 (Diameter 2: 4 mm)		Grade 3 (Diameter < 2 mm)	
			Number	Weight (g)	Number	Weight (g)	Number	Weight (g)
Hermes	**Control**		3.7 de	0.28 de	3.7 cd	0.09 ab	6 c	0.049 c
	Si-NPs	**200**	7 a	0.89 a	4.7 bc	0.17 a	6 c	0.08 b
		400	4.7 b-e	0.44 bcd	3.3 cde	0.121 ab	5.7 d	0.069 b
Charlotte	**Control**		3.3 e	0.32 cde	3.3 cde	0.122 ab	3 f	0.026 d
	Si-NPs	**200**	5 bcd	0.41 bcd	3.7 cd	0.09 ab	3.7 e	0.038 c
		400	5.3 bc	0.38 b-e	5.3 b	0.16 a	6 c	0.07 b
Inova	**Control**		6 ab	0.56 bc	5.3 b	0.17 a	11 a	0.12 a
	Si-NPs	**200**	6 ab	0.43 bcd	7 a	0.17 a	8 b	0.083 b
		400	5 bcd	0.38 b-e	5.3 b	0.15 a	8 b	0.083 b
Maris peer	**Control**		4.3 cde	0.17 e	2 e	0.04 b	1.3 g	0.011 e
	Si-NPs	**200**	4 cde	0.38 b-e	2.7 de	0.09 ab	3 f	0.039 e
		400	4.7 b-e	0.60 b	3.3 cde	0.15 a	3.7 e	0.045 c

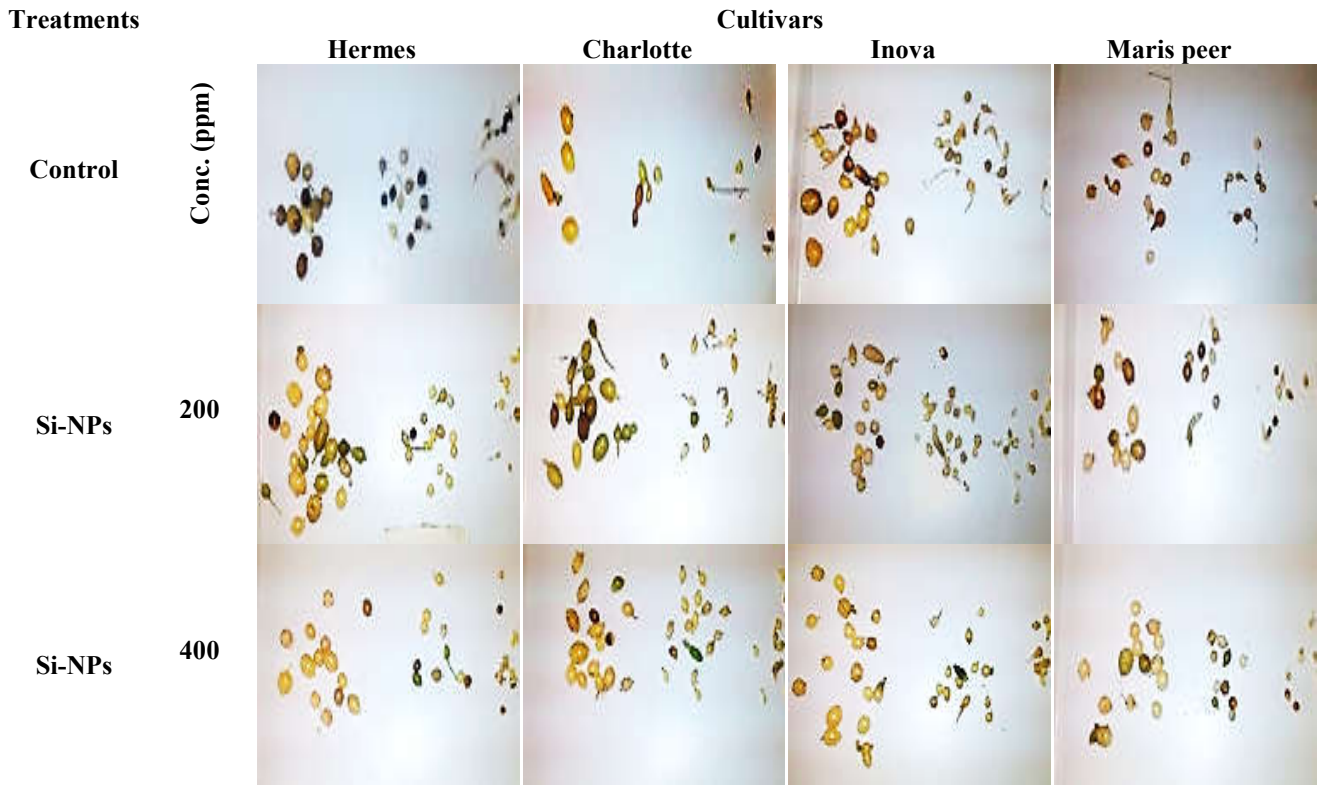

Fig. (1): Overall growth of microtubers of four cultivars at harvest after application of Si-NPs

Isoenzymes of POD in Shoots:

Eleven bands of POD isoenzymes were separated from shoots of all cultivars under study (Table 3 and Fig. 2). POD 4, 5, 6, 7, 8 and 9 isoenzymes bands were detected in all treatments as well as PEG-stressed explants with different range of high to low intensity. Si-NPs application influenced appearance or disappearance of POD isoenzymes bands. POD1 isoenzyme was absent in control explants, but found in both Si-NPs concentrations in all cultivars except Inova cv.. Also, POD 3 undetected in control explants, but found in both Si-NPs concentrations in all cultivars except Maris peer cv.. However, POD10 isoenzyme band was detected in control shoot but disappeared in Si-NPs at both concentrations in Charlotte and Inova cvs. Results were agreed with Veitch, (2004) who reported that, more than 70 isoenzymes of peroxidase were found in *Arabidopsis thaliana*, which induced by external factors such as wounding, stress, and pathogen attacks. Isoenzymes were differed one amino acid because of structural motifs or regions in genetic structure. Gowayed *et al.* (2017) reported that, the highest value of antioxidant activity of both Glutathione peroxidase was recorded at 50 ppm of SiO2-NPs in both potato Proventa and Sante cvs., under saline conditions.

Table (3): Ideogram of POD isoenzymes as affected by Si-NPs in shoot of potato plantlets under drought stress

Peroxidase isoforms	Relative mobility	Cultivars											
		Hermes			Charlotte			Inova			Maris peer		
		Treatments											
		Control	Si-nps 200 ppm	Si-nps 400 ppm	Control	Si-nps 200 ppm	Si-nps 400 ppm	Control	Si-nps 200 ppm	Si-nps 400 ppm	Control	Si-nps 200 ppm	Si-nps 400 ppm
POD 1	**0.20**	-	+	+	-	+	+	+	+	+	-	+	+
POD 2	**0.25**	-	+	+	+	+	+	+	+	++	+	+	+
POD 3	**0.30**	-	++	++	-	++	++	-	++	++	+	++	++
POD 4	**0.35**	++	++	++	++	++	++	++	++	++	++	++	++
POD 5	**0.40**	+	++	++	+	++	++	++	++	++	++	++	++
POD 6	**0.45**	++	++	++	++	++	++	++	++	++	++	++	++
POD 7	**0.50**	+	++	++	+	++	++	++	++	++	+	++	++
POD 8	**0.55**	+	++	++	+	++	++	+	++	++	+	++	++
POD 9	**0.60**	+	++	++	+	++	++	+	++	++	+	++	++
POD 10	**0.65**	+	++	++	+	-	-	+	-	-	+	++	++
POD 11	**0.70**	+	++	++	-	-	-	+	-	-	-	++	++

[(++): High intensity; **(+):** Low intensity; **(-):** Absent**].**

Isoenzymes of POD in Microtubers:

Also, as shown in shoots, eleven bands of POD isoenzymes were separated from microtubers of all cultivars under study (Table 4 and Fig. 2). POD 4, 5, 6, 7 and 8 isoenzymes were found in all treatments as well as PEG-stressed explants with different range of high to low intensity. POD 1 undetected in control explants but found in both Si-NPs concentrations in Hermes and Charlotte cvs. POD 10 was found in control explants but disappeared in high concentration (400 ppm) of Si-NPs in all cultivars under study. Results were coordinated with Préstamo and Manzano (1993), who isolated seven different isoforms of peroxidase isozymes with molecular weight 39, 40, 48, 55, 67, 84-94, 120-130 KDa from potato tubers using SDS-PAGE according to Laemmli (1970). They also reported that number of peroxidase isozymes was differed according to plant species and age of plant organ.

Table (4): Ideogram of POD isoenzymes as affected by Si-NPs in potato microtubers under drought stress

Peroxidase isoforms	Relative mobility	Cultivars											
		Hermes			Charlotte			Inova			Maris peer		
		Treatments											
		Control	Si-nps		Control	Si-nps		Control	Si-nps		Control	Si-nps	
			200 ppm	400 ppm		200 ppm	400 ppm		200 ppm	400 ppm		200 ppm	400 ppm
Pod 1	**0.20**	-	+	+	-	+	+	+	-	+	+	+	+
Pod 2	**0.25**	++	++	++	++	++	++	++	-	++	++	++	++
Pod 3	**0.30**	-	-	-	-	-	-	++	-	++	-	-	-
Pod 4	**0.35**	++	++	++	++	++	++	++	++	++	++	++	++
Pod 5	**0.40**	++	+	+	+	+	+	++	+	+	+	+	+
Pod 6	**0.45**	+	++	++	++	++	++	++	++	++	++	++	++
Pod 7	**0.50**	+	++	++	+	++	++	+	++	++	++	++	++
Pod 8	**0.55**	+	+	+	+	+	+	+	+	+	+	+	+
Pod 9	**0.60**	+	+	+	+	+	-	+	-	+	+	+	+
Pod 10	**0.65**	+	+	-	+	+	-	+	-	-	+	+	-
Pod 11	**0.70**	+	-	-	+	+	-	-	-	-	+	+	-

[(++): High intensity; **(+):** Low intensity; **(-):** Absent**]**

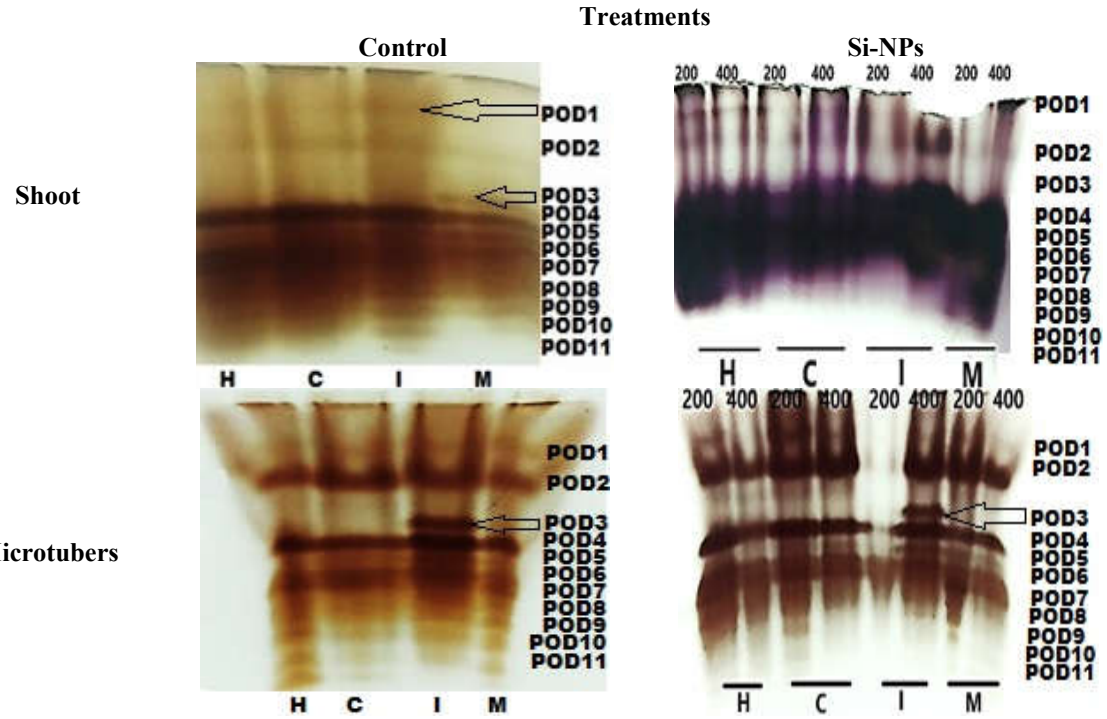

Fig. (2): Isoenzymes profile of peroxidases (POD) as affected by Si-NPs, in shoots and microtubers of four potato cultivars after 160 days of PEG (10%) exposure., **[H** (Hermes); **C** (Charlotte); **I** (Inova); **M** (Maris peer)**]**

Isoenzymes of PPO in Shoots:

Ten bands of PPO isoenzymes were separated from shoots of all cultivars under study (Table 5 and Fig. 3). PPO 1 and 2 isoenzymes undetected in shoots of all treatments as well as control. PPO 4, 6 and 7 isoenzymes were found in all treatments as well as PEG-stressed explants with different range of high to low intensity. PPO 8 and 9 were detected in control explants but disappeared in low Si-NPs concentration (200 ppm) in Inova and Maris peer cvs. PPO 10 was showed in control explants but disappeared in high Si-NPs concentration (400 ppm) in Hermes and Charlotte cvs. Multiple forms of PPO have been found in several plant species, for example four isoforms in apple (Harel *et al*., 1973), eight isoforms in grape (Sanchez-Ferrer *et al*., 1989). Also, Kowalski *et al.* (1993) found that, high activity of PPO had correlated with high resistance of potato against biotic stress. PPO oxidized the phenolic compound, p-hydroxyphenyl propionic acid, producing a cement-like substance on cell wall. These multiple forms exhibit distinct differences in their physicochemical and enzymatic properties. Despite the available evidence, there are some conflicting reports regarding the number of molecular forms of PPO in some species/tissue. Production of artifacts, inter conversion among the PPO forms, hormonal induction, and attachment of phenolic products or carbohydrates could result in multiple isoforms of PPO (Yoruk and Marshall, 2003). Tran *et al.* (2012) showed that, PPO responsible for plant defense and pigment formation, localized in thylakoid membrane of chloroplasts and oxide the phenolic compounds located in vacuoles to quinones. Lineage-specific expansion/duplication and gene loss are two important reasons for the extreme variability of size and structure observed in land plant PPO. However, five putative genes responsible for PPO isoforms in potato and eleven in soybeans (Taranto *et al,* 2017). Gowayed *et al.* (2017) obvious that, SiO_2-NPs increased the activity of antioxidant enzymes under saline conditions in both potato Proventa and Sante cvs.

Isoenzymes of PPO in Microtubers:

Also, as shown in shoots, ten bands of PPO isoenzymes were separated from microtubers of all cultivars under study (Table 6 and Fig. 3). PPO 4 and 5 isoenzymes were found in all treatments as well as PEG-stressed explants with different range of high to low intensity. PPO 8 was detected in control microtubers but disappeared in both Si-NPs concentrations in Inova and Hermes cvs. Thygesen *et al.* (1995) found that multiple PPO isoforms can be concomitantly present in potato tubers, due to the transcription of different genes. PPO activity was high in stolons, tubers, roots, and flowers but low in leaves and stems of potato. Its activity was increased during tuber development. The activity was greatest at the tuber exterior, including the skin and cortex tissue 1 to 2 mm beneath the skin. PPO isoforms differed according to subcellular organelles, stage of tissue development, attack by pathogen and treatment with hormones. More than 6 copies of PPO genes were located on the chromosome 8. PPO molecular weight was 15-20, 40-45 and 55-65 KDa. Elhamahmy *et al.* (2021) found that, number of isoenzymes of both POD or PPO may be increased or decreased according to potato cultivar (genetic structure) or induction of mutation with gamma rays under salt stress conditions.

Table (5): Ideogram of PPOs isoenzymes as affected by Si-NPs in potato shoot under drought stress

Polyphenol oxidase isoforms	Relative mobility	Cultivars											
		Hermes			Charlotte			Inova			Maris peer		
		Treatments											
		Control	Si-nps		Control	Si-nps		Control	Si-nps		Control	Si-nps	
			200 ppm	400 ppm		200 ppm	400 ppm		200 ppm	400 ppm		200 ppm	400 ppm
PPOs 1	**0.25**	-	-	-	-	-	-	-	-	-	-	-	-
PPOs 2	**0.30**	-	-	-	-	-	-	-	-	-	-	-	-
PPOs 3	**0.35**	-	-	-	-	-	-	+	+	+	-	-	-
PPOs 4	**0.40**	++	++	++	++	++	++	++	++	++	++	++	++
PPOs 5	**0.45**	+	-	-	+	-	-	++	+	+	+	-	-
PPOs 6	**0.50**	++	++	++	++	++	++	++	++	++	++	+	++
PPOs 7	**0.55**	+	++	+	+	++	++	+	++	++	+	+	++
PPOs 8	**0.60**	+	++	+	+	+	+	+	-	-	+	-	++
PPOs 9	**0.65**	+	++	+	+	-	-	+	-	-	+	-	++
PPOs 10	**0.70**	+	++	-	+	-	-	-	-	-	-	-	++

[(++): High intensity; **(+):** Low intensity; **(-):** Absent**].**

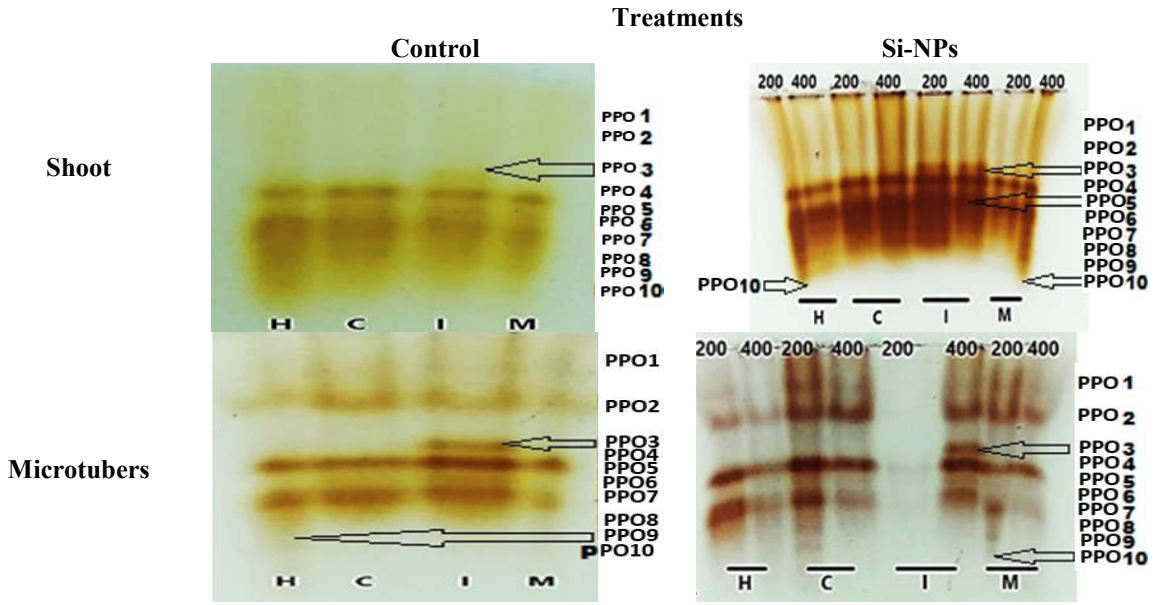

Fig. (3): Isoenzymes profile of polyphenol oxidase (PPOs) as affected by Si-NPs, in shoots and microtubers of four potato cultivars after 160 days of PEG (10%) exposure., **[H** (Hermes); **C** (Charlotte); **I** (Inova); **M** (Maris peer)**]**

Table (6): Ideogram of PPOs isoenzymes as affected by Si-NPs in microtubers of potato plantlets under drought stress

Polyphenol oxidase isoforms	Relative mobility	Cultivars											
		Hermes			Charlotte			Inova			Maris peer		
		Treatments											
		Control	Si-nps		Control	Si-nps		Control	Si-nps		Control	Si-nps	
			200 ppm	400 ppm		200 ppm	400 ppm		200 ppm	400 ppm		200 ppm	400 ppm
PPOs 1	**0.25**	-	+	-	-	+	+	-	-	+	-	+	+
PPOs 2	**0.30**	+	++	++	++	++	++	++	-	++	+	++	++
PPOs 3	**0.35**	-	-	-	-	-	-	++	-	++	-	-	-
PPOs 4	**0.40**	++	++	++	++	++	++	++	+	++	++	++	++
PPOs 5	**0.45**	+	++	+	+	++	+	++	+	++	+	++	+
PPOs 6	**0.50**	++	+	+	++	+	+	++	-	+	+	+	+
PPOs 7	**0.55**	++	+	-	++	+	+	++	-	+	+	+	+
PPOs 8	**0.60**	+	-	-	+	+	-	+	-	-	+	+	-
PPOs 9	**0.65**	+	-	-	-	+	-	-	-	-	-	+	-
PPOs 10	**0.70**	-	-	-	-	-	-	-	-	-	-	+	-

[(++): High intensity; **(+):** Low intensity; **(-):** Absent**].**

Molecular Characteristics of Five SCoT Primers of Shoots of Potato Explants Treated with Si-NPs

Amplification of specific bands was differed according to cultivars, SiNPs concentration and primers as shown in Table (7) and Fig. (4). 100 % of polymorphic bands among all potato cultivars and SiNPs treatments in shoots were detected by primer SCoT11, followed by SCoT 3 (67%). PEG-stressed shoots of Charlotte cv. gave the highest number of total monomorphic and polymorphic bands (28) compared to other treatment and cultivars. SCoT1 primer recorded the maximum number of total bands (10) with different base pairs (bp) compared to other primers. Bands with base pairs 385, 245, 180 with SCoT1, 540, 300, 240 with SCoT3, 395, 370, 300 with SCoT 6 and 300 with SCoT8 were monomorphic. Bands with 2630 and 2280bp amplified with SCoT 1 were found in control of Charlotte cv. only and undetected in both SiNPs treatments. Also, bands with 1500 and 1185bp amplified with SCoT 3 were found in control of Charlotte cv. only and unfound in both SiNPs treatments. However, bands with 450 and 400bp amplified with SCoT 3 disappeared in control of Charlotte cv. only and found in both SiNPs concentrations. Bands with 375bp amplified with SCoT 8 were appeared in control of Charlotte and Inova cvs. and undetected in high SiNPs concentration (400ppm). Bands with 645,470 and 400bp amplified with SCoT 11 disappeared in control of all cultivars and found in both SiNPs concentrations. Bands with 465, 300, 270 and 130bp amplified with SCoT 11 appeared in control of all cultivars and undetected in both SiNPs concentrations.

Table (7): Variation among Si-NPs treated and control shoots of potato cultivars in the number of bands of five SCoT primers

Primer name	Band no.	M.w (bp)	Cultivars: Hermes			Charlotte			Inova			Maris peer			Monomorphic bands	Polymorphic bands	Polymorphic %
			Treatments: Control	Si-NPs 200 ppm	Si-NPs 400 ppm	Control	Si-NPs 200 ppm	Si-NPs 400 ppm	Control	Si-NPs 200 ppm	Si-NPs 400 ppm	Control	Si-NPs 200 ppm	Si-NPs 400 ppm			
SCoT 1	1	2630	-	-	-	1	-	-	-	-	-	-	-	-			
	2	2280	-	-	-	1	-	-	-	-	-	-	-	-			
	3	1500	1	-	1	1	1	1	1	1	1	1	1	1			
	4	880	1	1	1	1	1	1	1	1	1	-	1	1			
	5	700	1	-	1	1	1	1	1	1	1	-	1	-			
	6	580	1	1	1	1	1	1	1	1	1	1	1	1			
	7	460	-	-	-	1	-	1	-	1	-	-	-	-			
	8	385	1	1	1	1	1	1	1	1	1	1	1	1			
	9	245	1	1	1	1	1	1	1	1	1	1	1	1			
	10	180	1	1	1	1	1	1	1	1	1	1	1	1			
	Total		**7**	**5**	**7**	**10**	**7**	**8**	**7**	**8**	**7**	**5**	**7**	**6**	**4**	**6**	**60**
SCoT 3	1	1500	-	-	-	1	-	-	-	-	-	-	-	-			
	2	1185	-	-	-	1	-	-	-	-	-	-	-	-			
	3	865	-	-	-	1	1	1	1	1	1	-	1	1			
	4	700	-	-	1	1	1	1	1	1	-	-	1	-			
	5	540	1	1	1	1	1	1	1	1	1	1	1	1			
	6	450	1	1	1	-	1	1	1	1	1	1	1	1			
	7	400	1	1	1	-	1	1	1	1	1	1	1	1			
	8	300	1	1	1	1	1	1	1	1	1	1	1	1			
	9	240	1	1	1	1	1	1	1	1	1	1	1	1			
	Total		**5**	**5**	**6**	**7**	**7**	**7**	**7**	**7**	**6**	**5**	**7**	**6**	**3**	**6**	**67**
SCoT 6	1	395	1	1	1	1	1	1	1	1	1	1	1	1			
	2	370	1	1	1	1	1	1	1	1	1	1	1	1			
	3	300	1	1	1	1	1	1	1	1	1	1	1	1			
	Total		**3**	**3**	**3**	**3**	**3**	**3**	**3**	**3**	**3**	**3**	**3**	**3**	**3**	**0**	**0**
SCoT 8	1	615	1	1	1	-	-	-	1	1	-	-	-	-			
	2	375	1	1	1	1	-	-	1	1	-	-	-	-			
	3	300	1	1	1	1	1	1	1	1	1	1	1	1			
	4	230	1	1	1	1	1	1	1	1	1	1	1				
	Total		**4**	**4**	**4**	**3**	**2**	**2**	**4**	**4**	**2**	**2**	**2**	**2**	**2**	**2**	**50**
SCoT 11	1	880	-	-	-	-	-	-	-	-	-	-	-	-			
	2	645	-	1	1	-	1	1	-	1	1	-	1	1			
	3	530	-	1	1	-	1	1	-	1	1	-	-	1			
	4	510	-	-	-	1	-	-	1	-	-	1	-	-			
	5	470	-	1	1	-	1	1	-	1	1	-	1	1			
	6	465	1	-	-	1	-	-	1			1	-	-			
	7	400	-	1	1	-	1	1	-	1	1	-	1	1			
	8	300	1	-	-	1	-	-	1	-	-	1	-	-			
	9	280	-	1	-	-	1	1	-	1	1	-	1	1			
	10	270	1	-	-	1	-	-	1	-	-	1	-	-			
	11	130	1	-	-	1	-	-	1	-	-	1	-	-			
	Total		**4**	**5**	**4**	**5**	**5**	**5**	**5**	**5**	**5**	**5**	**4**	**5**	**0**	**11**	**100**
Overall bands			**23**	**22**	**24**	**28**	**24**	**25**	**26**	**27**	**23**	**20**	**23**	**22**			

[(-): Absent; **(1):** High intensity**]**

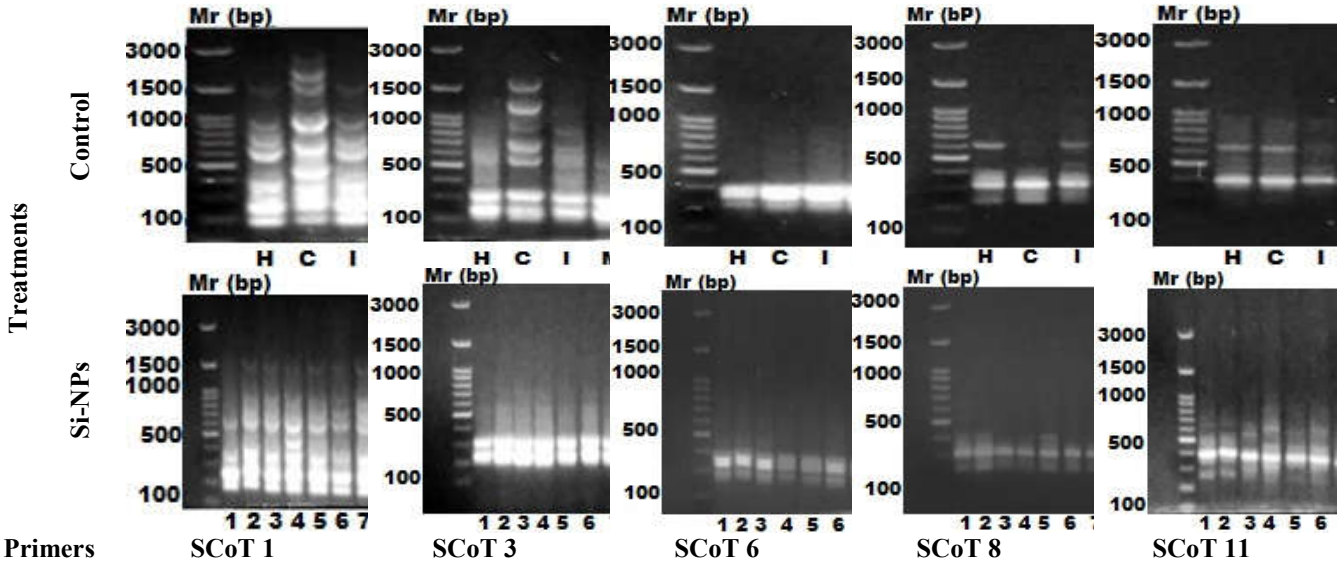

Fig. (4): SCoT amplification profile of Si-NPs treated shoots and control of four potato cultivars produced by 5 different primers as affected by drought stress. **[Mr** (marker)**];[H** (Hermes); **C** (Charlotte); **I** (Inova); **M** (Maris peer)**];[**in **Si-NPs** treatments, **1&2** (Hermes 200&400 ppm); **3&4**(Charlotte 200&400 ppm); **5&6** (Inova 200&400 ppm); **7&8** (Maris Peer 200&400 ppm)**]**

Results reported herein were agreed with Aversano *et al.* (2009),who found that ISSR markers was suitable tool for detecting genetic fidelity in *Solanum* species. Gorji *et al*. (2011) revealed that SCoT analysis was more effective in fingerprinting of potato varieties than other techniques as ISSR. Also, Tiwari *et al.* (2013), reported that ISSR was simple technique for detection of genetic stability of in vitro propagated potato microtubers. They also found 57 clear, distinct, and reproducible amplicons by 11 primers. Cluster analysis revealed 100 % of genetic similarity among the mother plant and its derivatives. Gupta *et al*. (2019) revealed that, SCoT marker was more efficient tool than ISSR, due to its highly polymorphic detection with *Capsicum* accessions.

Molecular Characteristics of Five SCoT Primers of Microtubers of Potato Explants Treated with Si-NPs

Table (8) and Fig. (5) showed 100% of polymorphic bands among all potato cultivars and SiNPs treatments in microtubers was detected by both primers SCoT3 and11. High Si-NPs treated microtubers of Hermes cv. gave the highest number of total monomorphic and polymorphic bands (23) compared to low SiNPs concentration and cultivars. SCoT3 primer recorded the maximum number of total bands (10) with different base pairs (bp) compared to other primers. Bands with base pairs 385, 245, 180 with SCoT1, 395, 370, 300 with SCoT6 were monomorphic. Band with 700bp amplified with SCoT 1 were found in control of Hermes and Charlotte cv. and undetected in both SiNPs treatments. Band with 580bp amplified with SCoT 1was found in control in all cultivars except Charlotte cv. and unfound in low SiNPs concentration. Band with 460bp amplified with SCoT 1was found in control of Hermes and Inova cvs. and unfound in high SiNPs concentration. Band with 540 bp amplified with SCoT 3was unfound in control of all cultivars and found in high SiNPs concentration. Band with 460bp amplified with SCoT 3was found in control of all cultivars except Charlotte cv. and unfound in all SiNPs concentrations. Bands with 385, 245 and 180bp amplified with SCoT 3 was found in control of all cultivars and unfound in all SiNPs concentrations. Bands with 450, 400, 300 and 240bp amplified with SCoT 3 was unfound in control of all cultivars and found in high SiNPs concentration. Bands with 470 and 400 bp amplified with SCoT 11 was undetected in control of all cultivars and found in all SiNPs concentrations. Band with 645bp amplified with SCoT 11was unfound in control of Charlotte and Inova cvs. and found in low SiNPs concentration. Band with 530bp amplified with SCoT 11was unfound in control of all cultivars except Maris peer cv. and found in both SiNPs concentrations. Bands with 510 and 300bp amplified with SCoT 11 was found in control of Hermes and Inova cvs. and unfound in both SiNPs concentrations. Band with 465bp amplified with SCoT 11 was found in control of all cultivars and unfound in both SiNPs concentration. Band with 280 bp amplified with SCoT 11 was unfound in control of Hermes and Inova cvs. and found in high SiNPs concentration.

CONCLUSION

High grade of microtubers formed by application of 200 ppm of SiNPs in Hermes and Inova cvs. Formation of high-grade of microtubers was attributed with presence or absence of specific isoenzymes of peroxidase, polyphenol oxidase and amplification of specific amplicons with different base pairs.

Table (8): Variation among Si-NPs treated and control microtubers of potato cultivars in the number of bands of five SCoT primers

Primer name	Band No.	M.W (bp)	Cultivars: Hermes			Charlotte			Inova			Maris peer			Monomorphic bands	Polymorphic bands	Polymorphic %
			Treatments: Control	Si-NPs 200 ppm	Si-NPs 400 ppm	Control	Si-NPs 200 ppm	Si-NPs 400 ppm	Control	Si-NPs 200 ppm	Si-NPs 400 ppm	Control	Si-NPs 200 ppm	Si-NPs 400 ppm			
SCoT 1	1	880	-	-	1	-	-	-	-	-	-	-	-	-			
	2	700	1	-	1	1	-	-	-	-	-	-	-	-			
	3	580	1	-	1	1	1	-	1	-	1	1	-	1			
	4	460	1	1	-	1	1	1	1	-	-	1	1	1			
	5	385	1	1	1	1	1	1	1	1	1	1	1	1			
	6	245	1	1	1	1	1	1	1	1	1	1	1	1			
	7	180	1	1	1	1	1	1	1	1	1	1	1	1			
	Total		6	4	6	6	5	4	5	3	4	5	4	5	3	4	57
SCoT 3	1	700	-	-	1	-	-	-	-	1	-	-	1	1			
	2	540	-	-	1	-	1	1	-	1	1	-	1	1			
	3	460	1	-	-	-	-	-	1	-	-	1	-	-			
	4	450	-	-	1	-	1	1	-	1	1	-	1	1			
	5	400	-	-	1	-	1	1	-	1	1	-	1	1			
	6	385	1	-	-	1	-	-	1	-	-	1	-	-			
	7	300	-	1	1	-	1	1	-	1	1	-	1	1			
	8	245	1	-	-	1	-	-	1	-	-	1	-	-			
	9	240	-	1	1	-	1	1	-	1	1	-	1	1			
	10	180	1	-	-	1	-	-	1	-	-	1	-	-			
	Total		4	2	6	3	5	5	4	6	5	4	6	6	0	10	100
SCoT 6	1	395	1	1	1	1	1	1	1	1	1	1	1	1			
	2	370	1	1	1	1	1	1	1	1	1	1	1	1			
	3	300	1	1	1	1	1	1	1	1	1	1	1	1			
	Total		3	3	3	3	3	3	3	3	3	3	3	3	3	0	0
SCoT 8	1	615	-	-	-	-	1	-	1	1	1	-	-	-			
	2	375	1	1	1	1	1	1	1	1	1	-	-	1			
	3	300	1	1	1	1	1	1	1	1	1	1	1	1			
	4	230	1	1	1	1	1	1	1	1	1	1	1	1			
	Total		3	3	3	3	4	3	4	4	4	2	2	3	2	2	50
SCoT 11	1	880	-	-	-	-	-	-	-	-	-	-	-	-			
	2	645	-	-	1	-	1	1	-	1	-	-	-	-			
	3	530	-	1	1	-	1	1	-	1	1	-	-	-			
	4	510	1	-	-	-	-	-	1	-	-	-	-	-			
	5	470	-	1	1	-	1	1	-	1	1	-	1	1			
	6	465	1	-	-	1	-	-	1	-	-	1	-	-			
	7	400	-	1	1	-	1	1	-	1	1	-	1	1			
	8	300	1	-	-	-	-	-	1	-	-	-	-	-			
	9	280	-	-	1	-	1	-	-	1	1	-	-	-			
	Total		3	3	5	1	5	4	3	5	4	1	2	2	0	9	100
Overall bands			19	15	23	16	22	19	19	21	20	15	17	19			

[(-): Absent; **(1):** High intensity

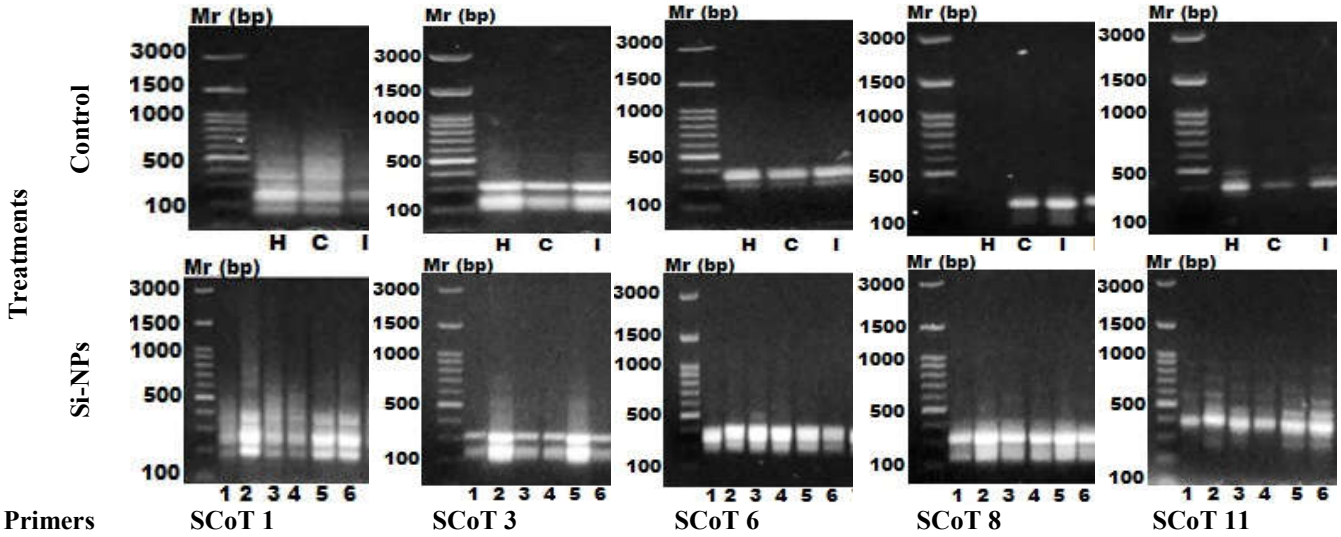

Fig. (5): SCoT amplification profile of Si-NPs treated microtubers and control of four potato cultivars produced by 5 different primers as affected by drought stress. **[Mr** (marker)**];[H** (Hermes); **C** (Charlotte); **I** (Inova); **M** (Maris peer)**];[**in **Si-NPs** treatments, **1&2** (Hermes 200&400 ppm); **3&4**(Charlotte 200&400 ppm); **5&6** (Inova 200&400 ppm); **7&8** (Maris Peer 200&400 ppm)**]**

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الصفات الجزيئية للنباتات الصغيرة للبطاطس المعاملة بجزيئات النانوسيليكون تحت ظروف الجفاف

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تم فحص تكوين رتب مختلفة من الدرنات الصغيرة في ٤ أصناف من البطاطس (هيرمس - شارلوت – انوفا - مارس بير) والمعاملة بتركيزين من النانوسيليكون ٢٠٠ و ٤٠٠ جزء في المليون تحت ظروف الجفاف والمستحث بواسطة البولي اثيلين جليكول بتركيز ١٠%. اظهر التركيز المنخفض من النانوسليكون أعلى عدد ووزن للدرنات الصغيرة للرتبة الأولى للدرنات الصغيرة (٧ و ٠.٨٩ جم على التوالي) في صنف الهرمس. ارتبطت النتائج بوجود المشابه الانزيمى الأول لإنزيم البيروكسيديز في الدرنات والمشابه الانزيمى الثامن للبولى فينول اوكسيديز في الدرنات والعاشر من نفس الإنزيم في الجزء الخضري. كما ارتبطت النتائج بظهور العدد الأقصى (٢٣) من الحزم المتشابهة والمختلفة في الدرنات تم نسخها بواسطة ٥ أنواع مختلفة من البريمرات (سكوت). كما اختفت الحزم ذات الوزن الجزيئى ٧٠٠ و ٤٦٠ زوج من القواعد مع سكوت ١ و٥١٠ زوج من القواعد مع سكوت ١١ وظهور ٦٤٥ و ٢٨٠ مع سكوت ١١ في الدرنات الصغيرة. أعطى نفس التركيز أعلى عدد ووزن (٧ و ٠.٦٧ جم على التوالي) للرتبة الثانية في صنف الانوفا. وارتبطت النتائج السابقة باختفاء المشابه الانزيمى العاشر للبيروكسيديز في الجزء الخضري واختفاء المشابه الانزيمى ٨ و ٩ للبولى فينول اوكسيديز في الجزء الخضري والمشابه الانزيمى ٨ لنفس الإنزيم في الدرنات. اختفاء الحزم ٣٧٥ زوج من القواعد المنسوخ بواسطة سكوت ٨ في الجزء الخضري. يمكن التوصية باستخدام التركيز المنخفض (٢٠٠ جزء في المليون) في إنتاج الرتب الكبيرة من الدرنات الصغيرة خاصة مع الصنف هيرمس و انوفا.