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Full Length Article

Improving the Salinity Tolerance in Potato (*Solanum tuberosum* L.) by Exogenous Application of Silicon Dioxide Nanoparticles

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Abstract

Salinity is one of the major abiotic stresses affecting potato growth and productivity in semi-arid and growing zones. An increase in salt tolerance by selecting superior potato cultivars using new biotechnology approaches and application of nanomaterial could improve the productivity and profitability of potato. In this study, two cultivars of potato were exposed to different treatments of NaCl at 50 and 100 mM and SiO_2-NPs at 50 and 100 mg L^{-1} under *in vitro* and greenhouse conditions. After 35 and 90 days of treatment, different growth parameters, protein analysis and antioxidant enzyme activities were examined. Addition of NaCl to the medium induced a significant decrease in most growth traits in comparison to control and SiO_2-NPs treatments with higher NaCl concentration (100 mM) having a more adverse effect on growth. Inclusion of different concentrations of SiO_2-NPs to the medium reduced the deleterious effect of salinity, which was more pronounced at 50 mg L^{-1} SiO_2-NPs than at 100 mg L^{-1}. A protein band was observed (22.39 kDa) which can be considered as positive marker for salt stress in cv. Sante and a novel band (70.412 kDa) corresponded to damaging mechanisms as a result of toxic effects in cv. Proventa. Cultivar Proventa was less affected by salt stress than cv. Sante. In conclusion application of SiO_2-NPs at 50 mg L^{-1} is the optimum dose to enhance and help improving *in vitro* plant growth and mitigating the negative effects of salinity in potato. © 2017 Friends Science Publishers

Keywords: Antioxidant enzymes; *In vitro* culture; Nano biotechnology; Potato; Protein polymorphism; Salinity; SiO_2-NPs
Abbreviations: 2.4-D −2,4-dichlorophenoxyacetic acid; dS − deci Siemens; BSA −bovine serum albumin; CFW −callus fresh weight; CRD −completely randomized design; GPX −glutathione peroxidase; CV −coefficient of variation; m −million; MS −Murashige and Skoog; MW - molecular weight; NPs −nanoparticles; NAA −naphthaleneacetic acid; Si−silicon; SDS-PAGE - sodium dodecyl sulphate-poly acrylamide gel electrophoresis; SOD −superoxide dismutases.

Introduction

Potato (*Solanum tuberosum* L.) as a vegetable crop is considered one of the most important crops in the world and is the fourth major world food crop after rice, wheat and corn in economic importance, and is used for human consumption, animal feed and as a source of starch for alcohol production. Potato covers a production area of about 19.3 million ha worldwide and yielded 368.00 million tonnes (FAO, 2012). The total area devoted for production in the year 2013 in Saudi Arabia was 16 thousand ha, with a total production of about 405.7 thousand tonnes (average 25.36 tonnes ha^{-1}) according to the Ministry of Agriculture, Saudi Arabia. In Saudi Arabia, about 133.7 million tonnes of tuber seeds were imported in the year 2012 for summer plantation from European countries. The tuber seed costs were about 6.8 million US Dollars.

Abiotic and biotic stresses remain serious stress constraints facing most crops including potatoes (Zaman *et al.,* 2015). The potato plant is considered moderately sensitive to salinity (Katerji *et al.,* 2000), where the leaves are the most sensitive part of the plant to salt (Bruns and Hecht-Buchholz, 1990). Van Hoorn *et al.* (1993) reported that under irrigated potato with 5.9 ds/m of salt a yield loss of up to 37% was incurred. Also, Rahman *et al.* (2008) showed that potato production is limited by high level of salt greater than 50 mM NaCl. The adverse effects of salinity stress on the potato plant can be considered as follow: (1) reduced growth of stem, leaves and tuber; (2) leaf chlorosis, tip burn and leaf burn; (3) restricted water uptake by root; (4) enhanced plant senescence; (5) reduced tuber yield and (6) browning and cracking of the tuber surface (Levy and Veilleux, 2007; Khenifi *et al.,* 2011; Jaarsma *et al.,* 2013).

Plant breeders aim to enhance plant salt tolerance by reducing the damaging effect of salt stress on plant growth and plant production through various different strategies. One of the recently experimented strategies is silicon

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application since its application has been found to be beneficial in increasing tolerance to abiotic stress (Epstein, 2009). Silicon can induce salt tolerance by a variety of methods including reducing Na+ uptake (Gong *et al.,* 2006), decreasing the concentration of malondialdehyde, the end-product of membrane lipid peroxidation (Liang *et al.,* 2003), increasing plant water content (Romero-Aranda *et al.,* 2006), increasing antioxidant enzyme activity (Zhu *et al.,* 2004), increasing the plasma membrane H^+-ATPase activity (Liang *et al.,* 2006), increasing photosynthesis activity (Liang *et al.,* 2007) and regulating biosynthesis of compatibile solutes (Zhu and Gong, 2014). Silicon can be supplied either as a bulk material (Guntzer *et al.,* 2012) or as a nano-particle (Tantawy *et al.,* 2015).

The development of nano-devices and nano-materials is proving to provide new applications in agriculture and plant biotechnology (Nair *et al.,* 2010; Reyna *et al.,* 2010). Nanomaterials have been shown to enhance faster plant germination/production, improve plant resistance to abiotic and biotic stress, assist efficient nutrient exploitation and promote plant growth, with reduced environmental impact compared to traditional approaches with bulk material (Reynolds, 2002). One of these nanoparticles signaling molecules is SiO_2, which has recently been shown to play an effective role in regulating diverse mechanisms involved in abiotic stress in plants (Manzer and Mohmed, 2014). Silicon nanoparticles possess greater surface area and higher solubility or surface reactivity compared to bulk material (Monica and Cremonini, 2009). Recently several studies have shown a significant effect of this nanotechnology to increase the value of agricultural production under salt stress (Tantway *et al.,* 2015), where silicon in two forms (regular 25%) and nano silicon (25%) were supplied through irrigation systems at concentrations of 4.0 and 5.0 $cm^3 L^{-1}$ for regular silicon and 1.0 and 2.0 $cm^3 L^{-1}$ for nano silicon. Data showed that most plant growth and yield parameters were improved under all silicon treatments compared to non-treated plants and concentration of 1.0 $cm^3 L^{-1}$ of nano silicon recorded the highest significant effect in mitigating the negative effects of salinity.

Potato breeding can play a role in improving varieties that can be grown under abiotic stress conditions. In plant-breeding program the selection of resistant cultivars in the field is considered important, however trial fields are commonly associated with variations in salt spatial distribution, irregular moisture availability and fluctuations of temperature through the growing season. This method also involves considerable space, time, labor, equipment and planting material resources (Arvin and Donnelly, 2008). The *in-vitro* method of tissue culture is a powerful tool for studying many trends of development and plant growth under controlled conditions (Shatnawi *et al.,* 2004) and *in-vitro* screening for salinity tolerance has been previously established (Potluri and Prasad, 1993; Zhang and Donnelly, 1997; Khenifi *et al.,* 2011). A large number of potato genotypes have now been commercialized through micro-tuber formation (Khrais *et al.,* 1998; Anoop and Chauhan, 2009).

Under salt stress, plants are exposed to extensive changes in their metabolism including enzymatic activities (Hasegawa *et al.,* 2000; Parida and Das, 2005), changes in protein shape and function (Chen and Tabaeizadeh, 1992; Barta and Batrova, 2008; Arefian *et al.,* 2014) and gene expression (Legay *et al.,* 2009) leading to a rise in the production of reactive oxygen species (ROS), which ultimately leads to a decrease in growth and increase in damage to the vegetative and productive part of the plants. In response to salt stress and to reduce the effect of oxidation, plants have evolved and developed various strategies of ROS scavenging such as superoxide dismutase (SOD) and glutathione peroxidase (GPX) and accumulation or depletion of certain metabolites resulting in alterations in gene expression and the levels of a small set of cellular proteins (Abu-Hena *et al.,* 2010). In most cases, antioxidant enzyme activity such as SOD increase in salt tolerant potato genotypes (Daneshmand *et al.,* 2010; Sajid and Faheem, 2014), however, occasionally the activity of SOD decreased with salinity stress (Zhang *et al.,* 2007).

SDS-PAGE of seed or leaf protein has been shown to be a practical biochemical technique and reliable method to detect the biochemical markers of lentil (Yu-Zbas *et al.,* 2008) and proving useful in selection of parental material for hybridization (Mennella *et al.,* 2001). The technique also reveals the complexity of the biochemical and physiological responses of plants to stress (Cooke, 1984; Chen and Tabaeizadeh, 1992; Arefian *et al.,* 2014). Bárta and Bártová (2008) reported that the electrophoretic protein profiles and their high stability and independence of ecological conditions could be used as genotype markers in potato.

The effectiveness of SiO_2-NPs to stimulate growth in some plants have been reported but there are no studies on the impact of the SiO_2-NPs on potato plants regenerated from tissue culture, therefor the objective of this study was to investigate the effectiveness of SiO_2-NPs in reducing the negative effect of salinity on potato plants *in-vitro.*

Material and Methods

An *In vitro*-regeneration and greenhouse acclimation system for potato was established at Genomic and Biotechnology Lab, Biological Science Department, University of Jeddah, Saudi Arabia during the period October 2015 to Jun 2016. Two different cultivars of potato tuber (*Solanium tuberosum* L.) namely Sante and Proventa were kindly provided by the Agriculture Research Center, Giza, Egypt for this study.

Preparation of SiO_2-NPs Suspension

Nanoparticles of SiO_2 with an average primary particle size of 50 nm with a corresponding surface area ranged from 200-600 $m^2 g^{-1}$ were purchased from the Nanotechnology Unit, Beni-Sueif University, Egypt. X-ray analysis and transmission electron microscopy (Fig. 1b) of the SiO_2-NPs

are presented in Fig. 1. Suspensions of SiO_2-NPs in concentration of 50 and 100 mg L^{-1} were prepared daily with deionized water and dispersed with a sonicator (JL-360, Shanghai, USA) for 30 min. The nanoparticle suspensions were centrifuged (3000 xg for 1 h) and filtered (0.7 µm glass filter) prior to being added to culture media (Helaly *et al.,* 2014).

In Vitro Regeneration of Potato

Growth and callus induction: Potato tubers were cut into small pieces, sown in quartz sands, irrigated with half strength Hoagland solution (Hogland and Arnon, 1983) and left to grow under greenhouse conditions at daily photoperiod and temperature of 12 h and 27°C, respectively. After 25-30 days of growth, 3 cm potato sprouts were washed with tap water for 5 min, then surface sterilized with 70-80% ethanol for 10 sec and washed with sterilized distilled water twice. Sprout segments were then dipped in commercial bleach solution (2.5% NaClO) with the addition to 5 drops of Tween-20 as a wetting agent, followed by rinsing thoroughly three times in sterilize distilled water, each for three minutes. After sterilization was completed, shoot tip explants (0.4 mm) were isolated under a binocular microscope under aseptic conditions in laminar air-flow hood and cultured *in –vitro* on a hormone-free growth medium (M1) composed of 4.4 g L^{-1} MS salts (Murashige and Skoog, 1962), 3% sucrose, 100 mg L^{-1} myo-inositol and 1.0 mg L^{-1} thiamin HCl. The media pH was adjusted to 5.7 before being solidified with 0.25% (w/v) Phytagel and autoclaving at 121°C for 20 min. Cultured explants were kept in an incubator at 25±2°C with 40 µmol m^{-2} s^{-1} cool white fluorescent light maintained in at 16 h photoperiod for 35 days until the formation of plantlet. Leaf explants were excised from 35-day-old plantlets, cut into halves with a sterile scalpel blade and placed abaxial side facing down on callus induction medium supplemented with 3.0 mg L^{-1} 2,4-D (2,4-dichlorophenoxyacetic acid) and 1.0 mg L^{-1} Kinetin according to Vijay *et al.* (2014) and incubated under same environmental conditions.

Nine experimental treatments were prepared (T1= control; T2= 50 mM NaCl; T3= 100 mM NaCl; T4= 50 mg L^{-1} SiO_2-NPs; T5= 100 mg L^{-1} SiO_2-NPs; T6= 50 mM NaCl + 50 mg L^{-1} SiO_2-NPs; T7= 50 mM NaCl + 100 mg L^{-1} SiO_2-NPs; T8= 100 mM NaCl + 50 mg L^{-1} SiO_2-NPs; T9= 100 mM NaCl + 100 mg L^{-1} SiO_2-NPs) and added to a hormone-free growth medium (M1) and callus proliferation medium (M2) prior to autoclaving. The jars were arranged in a completely randomized design (CRD) with 5 replicates in the incubator for five weeks. At the end of the incubation period the following traits were recorded: shoot length (SL), root length (RL), number of roots (NR), on the plantlets growing on hormone-free growth medium (M1), while callus fresh weight (CFW) and callus dry weight (CDW) were recorded on explants growing under callus proliferation medium (M2).

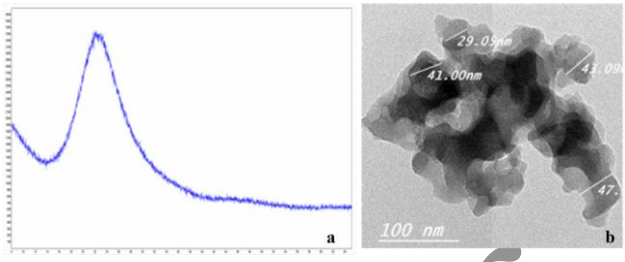

Fig. 1a: x-ray analysis and (**b**) transmission electron microscopy of SiO_2-NPs

Acclimation of *in-vitro* plantlets: Healthy plantlets were removed from glass jars and gently washed with tap water to remove culture medium attached to the roots and treated for 5 min. with (1.5 g L^{-1} Benlate) to inhibit any fungicide organisms. Plantlets were transferred to plastic pots containing sand + peatmoss, (1:1 v/v) and incubated in a growth room at 28°C and 16 h photoperiod. After two weeks the plants were transferred to a nethouse, covered with dark polyethylene bags which were gradually removed over two weeks by which time plants were acclimatized to *in-vivo* conditions. Pots were set up in rows and laid out in split plot combinations of treatments with three replicates. Different levels of NaCl and SiO_2-NPs treatments were applied as the main plots and potato cultivars were assigned as the subplots. SiO_2-NPs treatments were imposed as foliar sprays twice/week starting on 4 week-old *ex vitro* plants and ended 3 months later. Each treatment was represented by three pots each with three plants, giving a total of 27 plants per cultivar per treatment. Plants were irrigated with 3 (NaCl at 0, 50 and 100 mM) x 3 (SiO_2-NPs at 0, 50 and 100 mg L^{-1}) treatment combinations for 90 days. The plants were maintained at 22/16°C (day/night) under a relative humidity of 60% for the entire growth period. Plants were fertilized biweekly with a solution of 2 g L^{-1} 19/19/19 NPK fertilizer. The nutrient solution was freshly prepared at each application. The number of leaves (NL), number of branches (NB), number of minitubers (NT), tuber fresh weight (TFW), plant fresh weight (PFW) and plant dry weight (PDW) were recorded.

Biochemical Assays

Protein and antioxidant enzymes were assayed from young, fully expanded true leaves 2 days after the application of the last treatment and stored at -80ºC until biochemical analysis.

Protein Assay

Frozen leaves (0.25 g) were used to extract soluble protein according to Bradford (1976). SDS PAGE of leaf protein extracts were carried out in a vertical slab gel using 12% acrylamide according to Laemmli (1970) and a volume of 15-20 µ applied to each well. In a separate lane of the gel, a protein ladder ranging from 10 - 250 kDa (Thermo Fisher

Scientific, Waltham, MA, USA) was loaded as a marker. Electrophoresis was run in a protein II electrophoresis system (Bio-Rad, California, USA) for about one hour in running buffer at 150 V/100 mA. The gels obtained were photographed with a gel documentation system (Syngene, Cambridge, UK). The molecular weights of the dissociated or unknown protein bands were determined by using the standard curve obtained from the Rf-values and molecular weights of the protein ladder (10-250 KDa) and calculated using Gel Analyzer v.3 software.

GPX and SOD Antioxidant Enzyme Activities Assay

a mortar and pestle and kept in Eppendorf tubes at -80^{o}C Leaf tissues (0.5 g fresh weight) were ground in liquid nitrogen into a fine powder with until Biochemical investigation. Crude protein extracts were prepared by incubating the frozen powder with 0.1 mM EDTA, 0.1% TritonX-100, 1.0 mM C_2H_6O Sand 1% polyvinylpolypyrrolidone (PVPP), 50 mM Na_3PO_4 buffer (pH 0.7) and homogenized with a chilled mortar and pestle. The homogenized samples were centrifuged at 15000 xg for 15 min at 4ºC then aliquots of the supernatant were removed and used to determine enzyme activity. Protein concentration in crude extracts was adjusted to 0.5 mg mL^{-1}.

Glutathione peroxidase (EC 1.11.1.9) activity was assayed according to a modified method of Beyer and Fridovich (1987). 100 µL of 0.5 mg mL^{-1} crude extract was added to 1 mL reaction mixture tubes according to Roxas *et al.,* (1997). GPX activity was measured spectrophotometrically at 340 nm and expressed as µmol NADPH mg^{-1} protein. Total superoxide dismutase (EC 1.15.1.1) activity was assayed according to a modified method (Beyerad and Fridovich, 1987). Crude enzymatic extract (100 µL) was added to 3 mL of a reaction mixture as described by Dhindsa *et al.* (1981). SOD activity was measured spectrophotometrically at 560 nm and was expressed as U mg^{-1} protein.

Statistical Analysis

Analysis of variance (ANOVA) was applied to data using Costat Software (2006). Differences between individual means were estimated using Duncan's multiple range (Duncan, 1955). Coefficients of variation (CV) were calculated as indicators of variability within various measurements.

Results

Impact of NaCl and SiO_2-NPs on Different Growth Traits

ANOVA indicated that the influence of the main effects of the cultivars was significant ($p \leq 0.001$) only for shoot length, root length, number of roots, callus fresh weight, while for treatment effect either salinity or silicon significant difference was recorded for all the traits except number of tubers and plant dry weight (Table 1). The Coefficient of variation (CV) value ranged from 6.12% for number of leaf to 96.72% for plant dry weight, which indicated that many of the traits exhibited a wide range of variability. With regard to the salinity treatments cv. Proventa was more tolerant for most of the traits especially under the high dose of NaCl (100 mM) (Figs. 2-5). Also traits values were increased when treated under the lower concentration of SiO_2-NPs (50 mg L^{-1}) in both Proventa and Sante cultivars while the addition of SiO_2-NPs at higher concentration (100 mg L^{-1}) led to reductions in the mean value of all traits except plant dry weight in cv. Sante (0.415 g) compared to SiO_2-NPs at 50 mg L^{-1}. In response to NaCl in combination with SiO_2-NPs in cv. Proventa, the addition of SiO_2-NPs at 50 mg L^{-1} in combination with 50 mM NaCl or 100 mM NaCl, triggered a significant impact on salinity effects. In contrast, our results showed that SiO_2-NPs at 100 mg L^{-1} in combination with high or low NaCl had a toxic effect of potato plants and a decrease in all the traits values compared to control and others treatments was observed (Figs. 2-5).

Impact of NaCl and SiO_2-NPs on Protein Analysis

Using SDS-PAGE, protein bands were presented with molecular weights ranging from 16.702 kDa to 330.607 kDa (data not shown). Newly synthesized protein bands were observed for both NaCl and SiO_2-NPs treated cultivars, with 30 bands recorded in cv. Proventa and 29 in cv. Sante (Fig. 6). Both cultivars recorded similarity in the number of polymorphic bands (14 and 15) in Proventa and Sante respectively. From these results, it is clear that both cultivars performed similarly in their tolerance ability to salt stress and also their interaction with the various treatments via expression of stress resistance gene. The level of polymorphism was similar for both cultivar (Sante 55.172%, Proventa 46.667%) (Table 2). Under SiO_2-NPs treatments an increase in the number of bands was observed in both cultivars comparing with other treatments including control treatment (Fig. 6). This increase in the number of bands indicates that SiO_2-NPs application activated some genes which may be important protein associated with salt stress resistance. In cv. Sante, two bands at molecular weights of 278.071and 180.263 kDa were present only in SiO_2-NPs treatments (both 50 mg L^{-1} SiO_2-NPs and 100 mg L^{-1} SiO_2-NPs), also band at molecular weight 135.917 KDa was showed in all treatments except salt treatment 50 mM NaCl and 100 mM NaCl addition to treatment 50 mM NaCl + 50 mg L^{-1}SiO_2-NPs. Treatments 50 mM NaCl + 50 mg L^{-1} SiO_2-NPs; 50 mM NaCl + 100 mg L^{-1} SiO_2-NPs; 100 mM NaCl + 50 mg L^{-1} SiO_2-NPs; 100 mM + 100 mg L^{-1} SiO_2-NPs) exhibited a new band at 72.132 kDa. On the other hand, our results showed that bands at 45.88, 42.55 and 31.75 kDa were synthesized only under control and SiO_2-NPs (50 mg L^{-1} SiO_2-NPs and 100 mg L^{-1} SiO_2-NPs) in cv. Proventa.

Table 1: Summary of analysis of variance for different growth traits of growth and antioxidant enzymes activity of 2 cultivars of potato (*Solanum tuberosum* L.) under different treatments of NaCl and SiO_2-NPs

SV	df	MS												
		SL	N R	R L	N Lf	NB	NT	TF W	PFW	PDW	CFW	CDW	GPX	SOD
Cultivars (C.)	1	20.54***	2.24^{ns}	28.17***	0.91^{ns}	4.16^{ns}	3.13**	1.85*	1.13^{ns}	0.30^{ns}	1.03***	1.50*	84.50***	411.13***
Salinity (s)	2	175.32***	21.91***	40.67***	307.17***	5.02***	12.52***	3.06***	7.78***	0.15^{ns}	1.18***	0.002***	194.35***	1887.5***
Silicon (Si)	2	27.18***	13.46***	78.23***	96***	2.46**	0.69^{ns}	4.85***	6.96***	0.05^{ns}	0.80***	7.69***	41.60***	160.22***
genotype * Salinity	2	5.73**	8.13***	2.90**	0.91^{ns}	0.39^{ns}	4.96***	0.55^{ns}	1.11^{ns}	0.23^{ns}	0.23***	1.96**	0.88***	3.91^{ns}
genotype * Silicon	2	6.91**	1.13^{ns}	4.60***	1.41^{ns}	0.39^{ns}	1.69**	1.61*	3.98***	0.15^{ns}	0.33***	3.35^{ns}	0.37***	2.74^{ns}
Salinity * Silicon	4	2.03^{ns}	2.96**	32.53***	13.42***	0.38^{ns}	0.30^{ns}	1.46**	0.56^{ns}	0.11^{ns}	0.28***	6.74***	17.81***	153.47***
Error	40	0.97	0.59	0.37	0.90	0.42	0.27	0.33	0.41	0.14	0.01	3.26	0.04	4.39
Total	53													
CV%		16.25	18.20	10.39	6.12	30.15	23.51	39.68	17.11	96.72	13.96	9.83	2.09	1.82

*****Each column shows significant differences at $p \leq 0.05$ (*), $p \leq 0.01$(**), and $p \leq 0.001$ (***) between three-factor factorial (i) salinity, (ii) Si, (iii) potato cultivars by Duncan's multiple range test (DMRT); ns – non-significant difference. Also refer to abbreviations at the beginning of the article

Table 2: Monomorphic bands, polymorphic bands, total polypeptides bands and polymorphism percentage for the 2 different Potato (*Solanium tuberosum* L.) cultivars growing under different concentrations of NaCl and SiO_2-NPs

Cultivars	Sante	Proventa
Monomorphic bands	13	16
Polymorphic bands (without Unique)	15	14
Unique bands	1	0
Poly. + Uniq. Bands	16	14
Total number of bands	29	30
Polymorphism %	55.172	46.667
Mean of band frequency	0.714	0.821

Polymorphism % = Polymorphic bands\Total number of bands

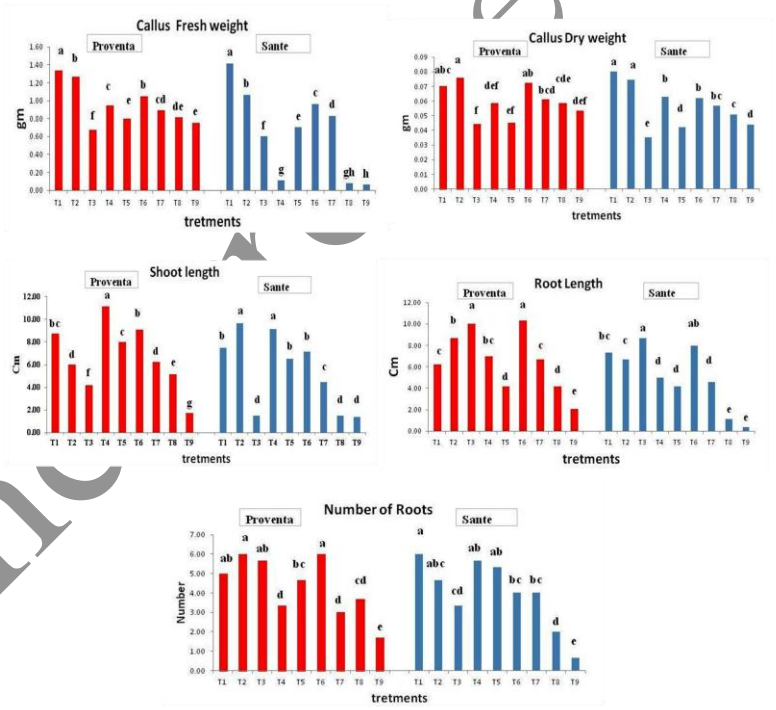

Fig. 2: Effect of NaCl and SiO_2-NPs on growth traits after 35 days *In vitro*. Data are represented as mean from three replicates. Different letters within each graph indicate statistically significant differences at $p \leq 0.05$ using Duncan's analysis

T1= control; T2= 50 mM NaCl; T3= 100 mM NaCl; T4= 50 mg L^{-1} SiO_2-NPs; T5= 100 mg L^{-1} SiO_2-NPs; T6= 50 mM NaCl + 50 mg L^{-1} SiO_2-NPs; T7= 50 mM NaCl + 100 mg L^{-1} SiO_2-NPs; T8= 100 mM NaCl + 50 mg L^{-1} SiO_2-NPs; T9= 100 mM NaCl + 100 mg L^{-1} SiO_2-NPs

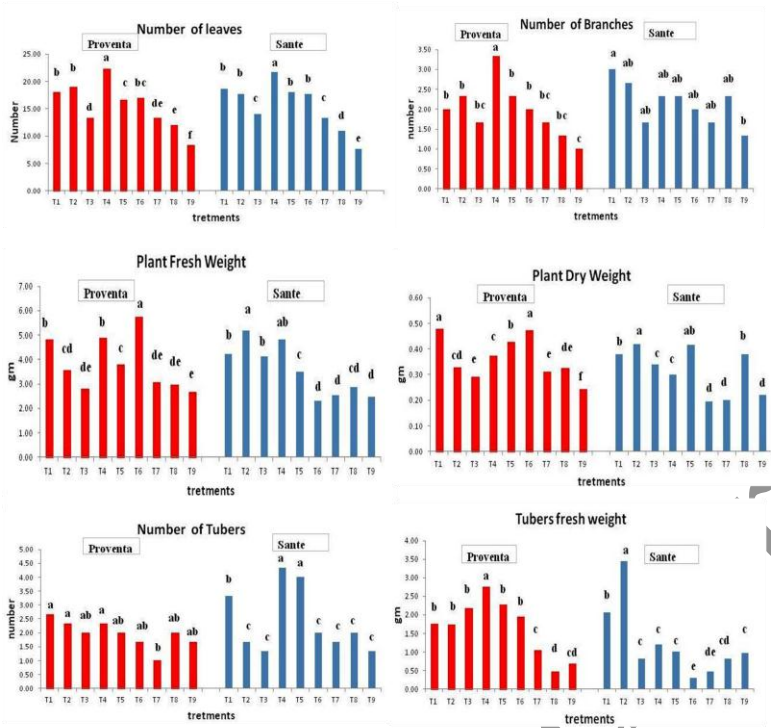

Fig. 3: Effect of NaCl and SiO_2-NPs on growth traits after 90 days in green house. Data are represented as mean from three replicates. Different letters within each graph indicate statistically significant differences at $p \leq 0.05$ using Duncan's analysis

T1= control; T2= 50 mM NaCl; T3= 100 mM NaCl; T4= 50 mg L^{-1} SiO_2-NPs; T5= 100 mg L^{-1} SiO_2-NPs; T6= 50 mM NaCl + 50 mg L^{-1} SiO_2-NPs; T7= 50 mM NaCl + 100 mg L^{-1} SiO_2-NPs; T8= 100 mM NaCl + 50 mg L^{-1} SiO_2-NPs; T9= 100 mM NaCl + 100 mg L^{-1} SiO_2-NPs

Also, Control treatment and 100 mM NaCl + 100 mg L^{-1} SiO_2-NPs showed no synthesis of new bands at 75.333 and 30.55 kDa, respectively, while these bands appeared in other treatments.

Impact of NaCl and SiO_2-NPs on GPX and SOD Antioxidant Enzyme Activities

Analysis of variance for GPX showed that the effect of genotypes, salinity, silicon and also the interaction effect between the three factor were highly significant, while for SOD only the interaction effect between genotype x salinity and genotype x silicon was non-significant (Table 3). Under salt treatment, GPX and SOD antioxidant activity showed lowered values compared to the control and the rest of the treatments (Table. 3). The highest value of antioxidant activity was recorded at 50 mg L^{-1} SiO_2-NPs but it was significantly reduced with the highest dose of SiO_2-NPs (100 mg L^{-1}) in both cv. Proventa and cv. Sante. Accumulation of GPX and SOD might be cultivar dependent, which may explain the inconsistent findings of the current research, where GPX activity was found to be highest in cv. Proventa (14.32, 8.17, 5.229, 15.006, 14.34, 13.19, 11.3, 10.97 and 7.99 µmol NADPH/mg protein) compared to cv. Sante (12.43, 5.68, 3.13, 12.57, 12.02, 9.88, 8.06, 8.31, 5.97 µmol NADPH/mg protein) at T1, T2, T3, T4, T5, T6, T7, T8 and T9, respectively, while SOD activity showed the opposite (Table 3). The plantlets treated with different doses of SiO_2-NPs (50 and 100 mg L^{-1}) in culture media reduced the effect of salinity by increasing the enzyme activity being more pronounced at the dose of 50 mg $L^{-1}$$SiO_2$-NPs than at 100 mg L^{-1} (Table. 3).

Table 3: Effect of different levels of NaCl and SiO_2-NPs on *GPX and SOD antioxidant enzyme activities* of two potato cultivars cv Sante and cv Proventa

Treatments	*antioxidant enzyme* GPX		*antioxidant enzyme* SOD	
	cv. Proventa	cv. Sante	cv. Proventa	cv. Sante
T1	14.32 ± 0.34^{b}	12.43 ± 0.12^{a}	124.00 ± 2.00^{a}	130.33 ± 2.52^{b}
T2	8.17 ± 0.15^{f}	5.68 ± 0.02^{e}	103.33 ± 1.15^{d}	110.33 ± 1.53^{e}
T3	5.29 ± 0.19^{g}	3.13 ± 0.08^{f}	99.00 ± 1.00^{e}	104.67 ± 1.15^{f}
T4	15.01 ± 0.09^{a}	12.57 ± 0.48^{a}	124.67 ± 1.15^{a}	134.00 ± 2.65^{a}
T5	14.34 ± 0.28^{b}	12.02 ± 0.08^{b}	122.67 ± 2.08^{a}	125.33 ± 1.53^{c}
T6	13.19 ± 0.07^{c}	9.88 ± 0.07^{c}	117.00 ± 1.00^{b}	117.67 ± 1.15^{d}
T7	11.30 ± 0.08^{d}	8.07 ± 0.29^{d}	106.00 ± 1.00^{d}	111.67 ± 2.08^{e}
T8	10.97 ± 0.11^{e}	8.32 ± 0.05^{d}	110.33 ± 2.31^{c}	118.33 ± 2.08^{d}
T9	7.99 ± 0.06^{f}	5.97 ± 0.03^{e}	104.67 ± 1.53^{d}	109.00 ± 1.00^{e}

T1= control; T2= 50 mM NaCl; T3= 100 mM NaCl; T4= 50 mg L^{-1} SiO_2-NPs; T5= 100 mg L^{-1} SiO_2-NPs; T6= 50 mM NaCl + 50 mg L^{-1} SiO_2-NPs; T7= 50 mM NaCl + 100 mg L^{-1} SiO_2-NPs; T8= 100 mM NaCl + 50 mg L^{-1} SiO_2-NPs; T9= 100 mM NaCl + 100 mg L^{-1} SiO_2-NPs

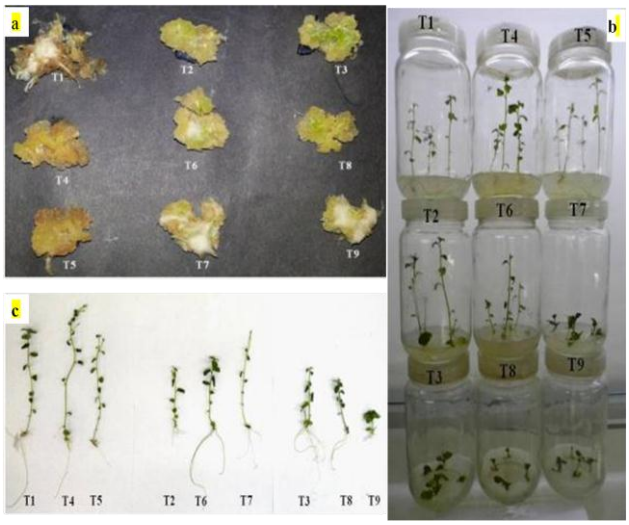

Fig. 4: Effect of NaCl and SiO_2-NPs on (a) callus growth: (b-c) seedling growth traits in cv. Proventa after 35 days of treatments

T1= control; T2= 50 mM NaCl; T3= 100 mM NaCl; T4= 50 mg L^{-1} SiO_2-NPs; T5= 100 mg L^{-1} SiO_2-NPs; T6= 50 mM NaCl + 50 mg L^{-1} SiO_2-NPs; T7= 50 mM NaCl + 100 mg L^{-1} SiO_2-NPs; T8= 100 mM NaCl + 50 mg L^{-1} SiO_2-NPs; T9= 100 mM NaCl + 100 mg L^{-1} SiO_2-NPs

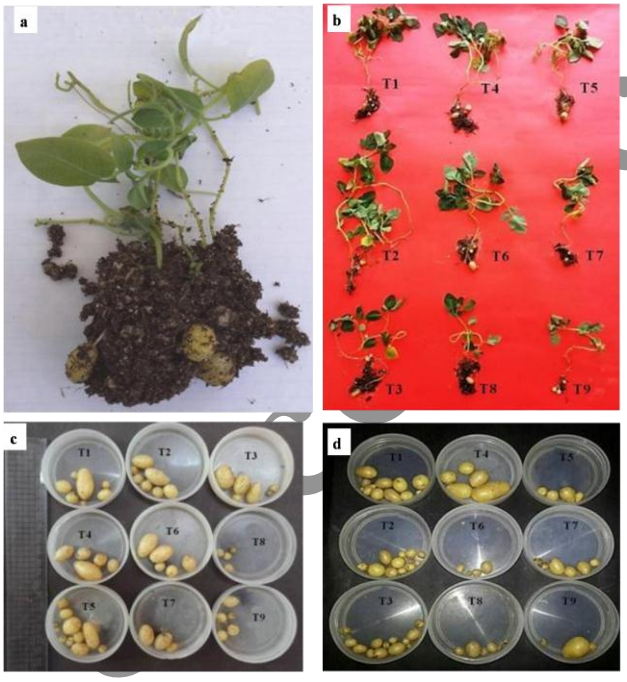

Fig. 5: Effect of NaCl and SiO_2-NPs on (a-b) plant growth on cv. Santa; (c) tuber growth in cv. Santa and d) tuber growth in cv. Proventa traits after 90 days of treatments

T1= control; T2= 50 mM NaCl; T3= 100 mM NaCl; T4= 50 mg L^{-1} SiO_2-NPs; T5= 100 mg L^{-1} SiO_2-NPs; T6= 50 mM NaCl + 50 mg L^{-1} SiO_2-NPs; T7= 50 mM NaCl + 100 mg L^{-1} SiO_2-NPs; T8= 100 mM NaCl + 50 mg L^{-1} SiO_2-NPs; T9= 100 mM NaCl + 100 mg L^{-1} SiO_2-NPs

Discussion

In order to determine the effectiveness of different dosages of SiO_2-NPs on potato growth under different salinity treatments, leaf explants were excised from 35-day-old plantlets and cultured *in vitro* before transfer to net house. The data showed that the addition of NaCl to medium drastically reduced all growth traits compared with control and SiO_2-NPs treatments and the higher NaCl concentration (100 mM) had a more detrimental effect that the lower level (50 mM) (Figs. 2-5). These reductions demonstrated how excess salt impairs water uptake, increase the accumulation of Na^+, Cl^- and counteract the uptake of essential nutrients thereby reducing growth and development (Tuna *et al.,* 2007). Also, under high concentration of salinity the destruction of the membrane structure inevitably disrupted ion homeostasis, affecting osmotic potential and inducing ion toxicity (Zhang *et al.,* 2014). Bruns and HechtBuchholz (1990) found that the salt-induced changes were mainly observed in the chloroplasts, especially in the thylakoids. Although the ultrastructural alterations induced by saline have been reported in many plant cells, but information regarding the effects of salinity on potato cells cultured in vitro is not specified and is incomplete (Gao *et al.,* 2015). The trait measured under the current study were increased when treated with the SiO_2-NPs under the lower concentration of SiO_2-NPs (50 mg L^{-1}) compared to the higher level (100 mg L^{-1}) and also the addition of SiO_2-NPs at 50 mg L^{-1} in combination with 50 mM NaCl or 100 mM NaCl triggered a significant beneficial impact to salinity treatment on some plant growth traits. In contrast SiO_2-NPs at 100 mg L^{-1} in combination with 50 mM NaCl or 100 mM NaCl had a toxic effect and a decrease in all the traits compared to control and others treatments. It has been suggested that plants under silicon treatment may strengthen plant defense against salt stress by osmotic adjustment and mitigation of ion toxicity by limiting Na^+ ion transportation (Yin *et al.,* 2013). Also, it has been shown that silicon treatment can increase the levels of gibberellic acid in the cells and have a plant hormone-like property that might play a vital role in cell division and consequently increased elongation (Soundararajan *et al.,* 2014). In other studies the application of Si-NPs increased the rate of photosynthesis, mesophyll conductance and plant water use efficiency of plants under saline stress (Tuna *et al.,* 2008; Haghighi and Pessarakli, 2013; Almutari, 2016). In previous reports, the identical alleviation of the adverse effects of salt stress was also observed with SiO_2-NPs treatment with reduced degradation of chlorophyll and increased net photosynthetic rate, stomatal conductance, transpiration rate, and water use efficiency (Siddiqui *et al.*, 2014).

Using SDS-PAGE, the results showed that cv. Proventa recorded highest number of bands (24 bands) compared with cv. Sante (14 bands) under salinity treatment (Table 2). Although, cv. Proventa was less affected by salt stress than cv. Sante, a higher level of

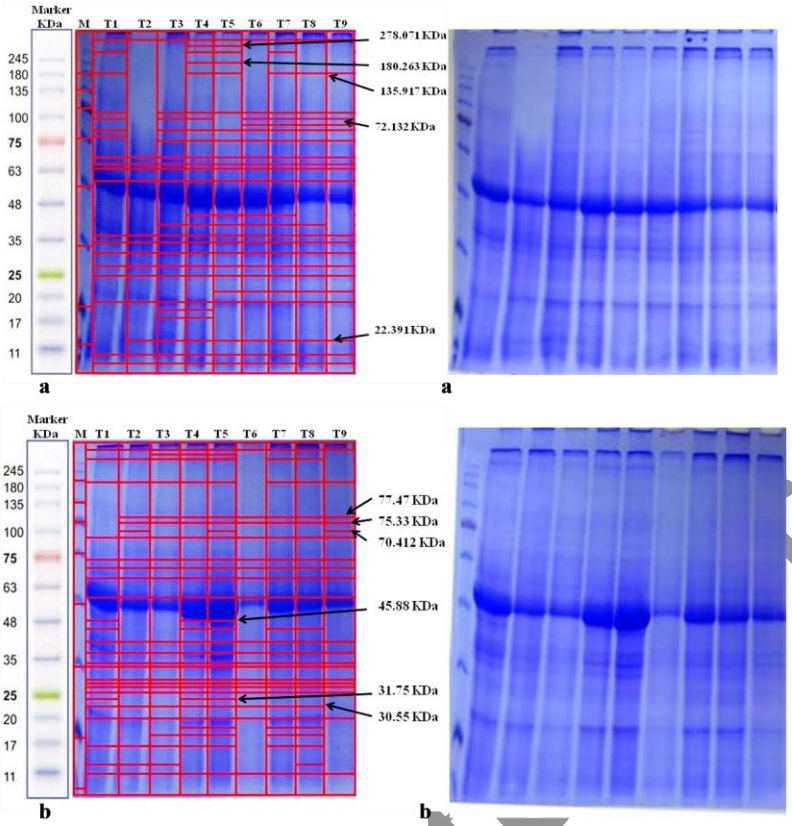

Fig. 6: SDS-PAGE protein patterns for two potato cultivars (a) Sante and (b) Proventa in response to NaCl and SiO_2-NPs
T1= control; T2= 50 mM NaCl; T3= 100 mM NaCl; T4= 50 mg L^{-1} SiO_2-NPs; T5= 100 mg L^{-1} SiO_2-NPs; T6= 50 mM NaCl + 50 mg L^{-1} SiO_2-NPs; T7= 50 mM NaCl + 100 mg L^{-1} SiO_2-NPs; T8= 100 mM NaCl + 50 mg L^{-1} SiO_2-NPs; T9= 100 mM NaCl + 100 mg L^{-1} SiO_2-NPs

polymorphism (55.172%) was recorded for cv. Sante than cv. Proventa (46.667%). Our results indicate that both cv. Proventa and cv. Sante can be considered as tolerant cultivars but cv. Proventa was more tolerant than cv. Sante. An increase in the number of bands was evident when SiO_2-NPs were applied (Fig. 6). This may be the result of the activation of some genes and consequently the production of important protein associated with salt stress responses. In our experiment the cv. Sante showed a new protein band at molecular weight 22.391 kDa which existed only under salinity treatment and SiO_2-NPs treatment as well as salinity together with SiO_2-NPs treatments and can be considered as a positive marker for stress (Fig. 6a). While, in cv. Proventa under treatments 50 mM NaCl, 100 mg L^{-1} SiO_2-NPs and 100 mM NaCl + 100 mg L^{-1} SiO_2-NPs a new band at molecular weight 70.412 kDa was generated, this protein band could correspond to the damage response mechanism as a result of toxic effect of NaCl at 50 mM or SiO_2-NPs at 100 mg L^{-1} (Fig. 6b). This may be due to NaCl may block the pathway of some gene, consequently, denaturing of enzymes involved in amino acid and protein that is involved in protecting the plants against environmental stress. The stress proteins may be function as a hydroxyl radical scavenger, or serves as energy and nitrogen source, stabilizes cell membranes by interacting with phospholipids (Aspinall and Paleg, 1981). Pareek *et al*. (1997) suggested that stress proteins could be used as important molecular markers for improvement of salt tolerance in plant breeding programmes.

Highly significant differences for GPX and SOD antioxidant enzymes was recorded between cultivars and treatments, which indicated that genotypic differences in response to salt and silicon nanoparticles dosages do not have similar trends to the genotypic differences under control conditions for most of traits measured in this investigation. Decrease of SOD and GPX activities under NaCl stress was recorded which can be explained as an inhibition of nitrogen uptake which then upregulate peptide biosynthesis of GPX and SOD antioxidant enzyme via inhibition of the genes encoding enzymes (Khodary, 2004). Gao *et al.* (2015) showed that the activities of SOD in leaves of potato plantlets significantly increased with the increase of NaCl concentration (0~100 mM) in medium. When exposed to 200 mM NaCl, especially after 6 weeks,

leaf cells were severely damaged, leading to the damage of cellular structure or alterations of metabolism, and reducing the synthesis of SOD. Highest value of antioxidant activity was observed at 50 mg L^{-1} SiO_2-NPs in both cultivars. A possible explanation for this is that the low and/or appropriate dose of SiO_2-NPs has a physiological and biochemical affect such as decreasing Na uptake, enhancing absorption of essential nutrients, acting as a delivering agent that delivers DNA, modifying of osmolytes and phytohormones or physical affect such as deposition on leaf apoplast that leads to a decrease in transpiration (Torney *et al.*, 2007; Rizwan *et al.*, 2015). Significant increase in GPX and SOD activity in response to salinity in combination with SiO_2-NPs compared to salinity stress at 50 mM NaCl and 100 mM NaCl was reported. This significant increase can appear to be scavenging of phospholipid hydroperoxides and thereby the protection of cell membranes from peroxidative damage (Srinieng *et al.*, 2015) and improvement in the ability of plant tissues to scavenge O_2 radicals, which might lessen membrane damage in the hypohydrated state (Murshed *et al.*, 2014).

Conclusion

The outcomes of this study provide that application of SiO_2-NPs at the appropriate dose rate significantly enhanced growth traits in salt-stressed potato plants and potato plantlets adapt to salt stress to some extent through increasing the activities of antioxidant enzymes, such as GPX and SOD. An increase in the number of protein bands in SiO_2-NPs was recorded and may be result from SiO_2-NPs activation of some genes and consequent expression of important proteins associated with salt stress responses. We recommend using SiO_2-NPs at 50 mg L^{-1} as optimized dose to improve plant growth under salinity stress.

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