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INFLUENCE OF NANO ZINC OXIDE ON THE *IN VITRO* CALLUS GROWTH, *EX VITRO* TUBER YIELD AND NUTRIONAL QUALITY OF POTATO (*Solanum tuberosum* L.) CULTIVARS UNDER SALT STRESS

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ABSTRACT

Potato (*Solanum tuberosum* L.) is one of the leading vegetable crops around the world. However, its growth, yield, and quality are reduced by several abiotic stresses such as salt stress. This study, consisted of two independent experiments was conducted to examine the effects of salt stress in potato and influence of nanoparticle-sized zinc (ZnO-NPs) in improving salt tolerance. The first experiment was conducted under in vitro conditions to evaluate the regenerated callus growth in response to the presence or absence of ZnO-NPs under different levels of NaCl (0, 17, 34, 51 and 68 mM). The second experiment was conducted using in vitro derived plantlets treated ex vitro with ZnO-NPs to evaluate its effect on tuber yield and nutritional quality under salt stress (0.0, 1.0, 2.0, 3.0 and 4.0 g L^{-1}). In both experiments, ZnO-NPs were applied at 0.0 or 50 mg L^{-1} to three potatoes cvs. Spunta, Nicola, and Hermes raised under five levels of NaCl-induced salt stress: 0, 17, 34, 51 and 68 mM. Salt stress significantly reduced the in vitro callus growth progressively with increasing NaCl levels, while ZnO-NPs had a positive impact on growth under salt stress. Potato tubers harvested ex vitro differed significantly for calcium and iron under different levels of salt stress in response to ZnO-NPs application. The number of tubers responded negatively to increasing levels of NaCl with or without ZnO-NPs application. All three potato cultivars, grown under 34 mM NaCl, regardless of ZnO-NPs application, had a significantly higher number of tubers than when grown at other NaCl concentrations. The highest number of tubers was recorded for all three tested potato cultivars receiving ZnO-NPs under non-saline conditions, whereas the lowest number was recorded in plants under high levels of salinity, with or without ZnO-NPs. The cv. Spunta was the least affected by salt stress followed by the cv. Hermis and cv. Nicola for most traits. Application of ZnO-NPs proved effective in ameliorating the salinity-induced harmful effects on yield and quality of potato.

Keywords: Potato, nanoparticles, NaCl, meristem culture, calcium, iron, protein

Abbreviation: $AdSO_4$ = Adenine sulfate; CFW = Callus fresh weight; BA = Benzyl adenine; GA_3 = Gibberellic acid; MS = Mushige and Skoog's medium; NAA = Napthalene acetic acid; NaCl = Sodium chloride; NPs = Nanoparticles; ZnO = Zinc oxide; PSi: Pound per square inch; Calcium (Ca); Iron (Fe).

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INTRODUCTION

Potato (*Solanum tuberosum* L.) is one of the leading vegetable crops grown and consumed worldwide (Liao *et al*., 2016). However, its sustainable production and quality are constrained by several challenges including temperature extremes drought, and salinity (Hijmans 2003; Levy *et al*., 2013). Among these, salt stress is one of the major abiotic stresses which limits the growth and productivity of all the crops including potato (Jaarsma *et al*., 2013). In general, potato is considered as moderately sensitive to salt stress (3-4 g L^{-1}) (Batelli *et al*., 2012). However, there exists genotypic variation, among potato genotypes, to salt stress. For instance, potato cv. Draja is moderately sensitive to salt stress whereas cv. Sponta is salt tolerant and cv. Diamont is salt sensitive (Jaarsma *et al*., 2013).

Salt stress disturbs the plant water relations and causes reduction in plant photosynthesis performance (Akhtar *et al*., 2015). At the cellular level, salt stress increases the cell vacuolization, causes swelling of the thylakoid membrane, and reduces the stacking of grana (Fidalgo *et al*., 2004). These changes affect the yield and quality of economic plant parts (Farooq *et al*., 2017). Although potato genotypes may vary in their response to salinity (Aghaei *et al*., 2009), a linear decrease was observed in most of growth and chemical traits such as shoot length, fresh and dry weight, and K+ content,

respectively in all genotypes with the increase in severity of salt stress (Ahmed *et al*., 2020).

In addition to carbohydrates, potato tubers also contain dietary proteins and fair amounts of minerals (Martínez-Ballesta *et al*., 2010). The potato tuber dietary protein helps to reduce blood cholesterol by increasing the circulation of cholesterol levels (Seo *et al*., 2014; Gambuti *et al*., 2016). However, drought stress may result in significant ($P < 0.0001$) increase in tuber soluble protein (Wegener *et al*., 2015). Potato tuber has a higher concentration of iron 41.09 mg kg−1 dry weight and traces of calcium 4.3 mg kg−1 dry weight (Gąsiorowska *et al*., 2018). However, Farooq *et al*. (2017) noticed that salt stress may reduce the uptake of micronutrients due to stronger competition by salt cations at the root surface.

Zinc (Zn) is one of the essential microelements and plays a key role in carbohydrate metabolism, protein biosynthesis, and gene expression related to abiotic stresses (Rehman *et al*., 2018a; Rossi *et al*., 2014). Plants raised from seed with high intrinsic Zn can grow well and yield better under less than optimum conditions (Faran *et al*., 2019). An adequate supply of Zn enables plants to tolerate abiotic stresses (Rehman *et al*., 2019). External Zn application may help improve nutrient uptake and modulate the key enzymes involved in plant metabolism (Aktas *et al*., 2006). The use of nanoparticles (NPs) is a novel approach in plant micronutrition (Liu and Lal 2015). Application of NP (1-100 nm) fertilizers helps to improve the nutrient absorption, translocation, and use efficiency compared to traditional fertilizers of non-nanoparticles sizes (Usman *et al*., 2020). Therefore, the application of Zn-containing nanoparticles can help in improving Zn availability. Application of Zn as ZnO-NPs has been very effective in improving the germination, metabolism, and growth of some vegetable crops such as cabbage, cauliflower, habanera peppers, basil and tomato (García-López *et al*., 2019).

The application of tissue culture has gained much momentum in plant improvement programs and commercial scale plant propagation under optimal and less than optimum conditions (Gu *et al*., 2004). Under salt stress, tissue culture technique may help to isolate salt-tolerant cells from successfully regenerated (Lutts *et al*., 2004; Bündig *et al*., 2017; Gowayed *et al*., 2017). The technique would allow mass screening of genotypes in a very limited space and time.

Zinc application helps improving salt tolerance in crop plants including potato (Aktas *et al*., 2006; Mahmoud *et al*., 2020). However, Zn application as NPs was found more effective than other forms of Zn (Hussein and Abou-Baker 2008). To the best of our knowledge, the influence of ZnO-NPs on the in vitro growth, and subsequent ex vitro tuber yield and quality of potato under salt stress has not been investigated. This study was, therefore, conducted to investigate the influence of ZnO-NPs on in vitro growth and in vivo tuber yield and quality of three potato cultivars under NaCl-induced salt stress.

MATERIALS AND METHODS

Plant materials and experimental conditions: Virus-indexed potato tubers of cultivars Spunta, Nicola, and Hermes, collected from Agricultural Research Center, Egyptian Ministry of Agriculture, Cairo, Egypt. The experiments were conducted during the period of July 2018 to May 20219 at Tissue Culture Laboratory, SCU, Egypt and Nutrition Lab, KAU, Saudi Arabia. Seeds were allowed to sprout in clay pots filled with wetted Vermiculite in a greenhouse. Sprouts (3 cm long) were taken, and surface sterilized with 10% Clorox for 5 min followed by washing three times with sterilized water. Meristem tip explants (0.5 mm) were isolated under a binocular and were cultured in test tubes containing 10 mL agar-solidified (7.0 g L^{-1}) MS medium (Murashige and Skoog 1962) containing basal salts, vitamins and supplemented with sucrose (3%). One shoot tip explant was cultured per tub, using 20 culture test tubes per each potato cultivar to obtain enough plantlets for further experiment. Medium pH was adjusted to 5.7 before the addition of agar, and the medium was autoclaved for 20 min at 121°C and 15 psi pressure. Cultures were incubated at 24 °C under cool-white fluorescent lamps (45 µmol m^{-2} s^{-1} at culture level) under a 16-h photoperiod for the development of plantlets.

Preparation of nano-ZnO particle suspension: Nanoparticles of ZnO with size of 30 nm, were purchased from Sigma–Aldrich Company, California, USA. For the preparation of nano-ZnO particle suspension, with concentrations of 50 mg L^{-1}, 1.5 g ZnO-NPs was dissolved in a liter of distilled water following to Kim *et al*. (2017). Good dissolution and homogeneity of the solution were ensured using a sonicator. The nanoparticle suspensions were centrifuged at 3000 × *g* under cooling (-4^0C) for one hour and then filtered using 0.7 µm glass filter. The filtrate was used for addition to the culture media.

Embryogenic callus and experimental details: Leaf disk explants (5 × 5 mm) from the fourth leaves (Fig. 1) of the previously formed potato plantlets were in vitro cultured on MS medium supplemented with sucrose (3%), benzyl adenine (BA; 3.0 mg L^{-1}) + naphthaleneacetic acid (NAA; 2.0 mg L^{-1}) for callus induction (Kumlay and Ercisli 2015). Media were adjusted for pH at 5.7, solidified with 7.0 g L^{-1} agar, and sterilized by autoclaving for 20 min at 121°C and 15 psi pressure. Cultures were incubated at 25°C in the dark for one week, followed by 4 weeks at 16 h photoperiod using cool white fluorescent light (45 µmol m^{-2} s^{-1}). The medium was refreshed by subculture the calli every 12 days to maintain the presence and effectiveness of ZnO-

NPs. After 5 weeks the soft, friable and light-yellow primary calli (about 1 cm in diameter) induced from the previous experiment were subcultured into fresh MS medium supplemented with gibberellic acid (GA3; 1.0 mg L^{-1}) + adenine sulfate (AdSO4; 20 mg L^{-1}) + BA (2.0 mg L^{-1}) for the induction of embryonic callus under the influence of ZnO-NPs and NaCl stress following Khatri *et al*. (2019) with minor modification. For this aim, medium treatments were: T1=0.0 mM NaCl; T2=17.00 mM NaCl; T3=34.00 mM NaCl; T4=51.00 mM NaCl; T5=68.00 mM NaCl; T6= 50.00 mg L^{-1} ZnO-NPs; T7= 17.00 mM NaCl+50.00 mg L^{-1} ZnO-NPs; T8= 34.00 mM NaCl +50.00 mg L^{-1} ZnO-NPs; T9= 51.00 mM NaCl+50.00 mg L^{-1} ZnO-NPs; T10= 68.00 mM NaCl +50.00 mg L^{-1} ZnO-NPs. The choice of these gradients in concentrations of NaCl was based on previous studies (Aghaei *et al*. 2008) and pre-experiments. Media preparation and culture incubation conditions were the same as described above. Jars were arranged in a growth room in a factorial completely randomized design (CRD). There were 8 jars per treatment, each jar representing a replication, with a total 80 jars. Seven weeks later, regenerated callus mass/treatment was assessed randomly to record the callus fresh and dry weights.

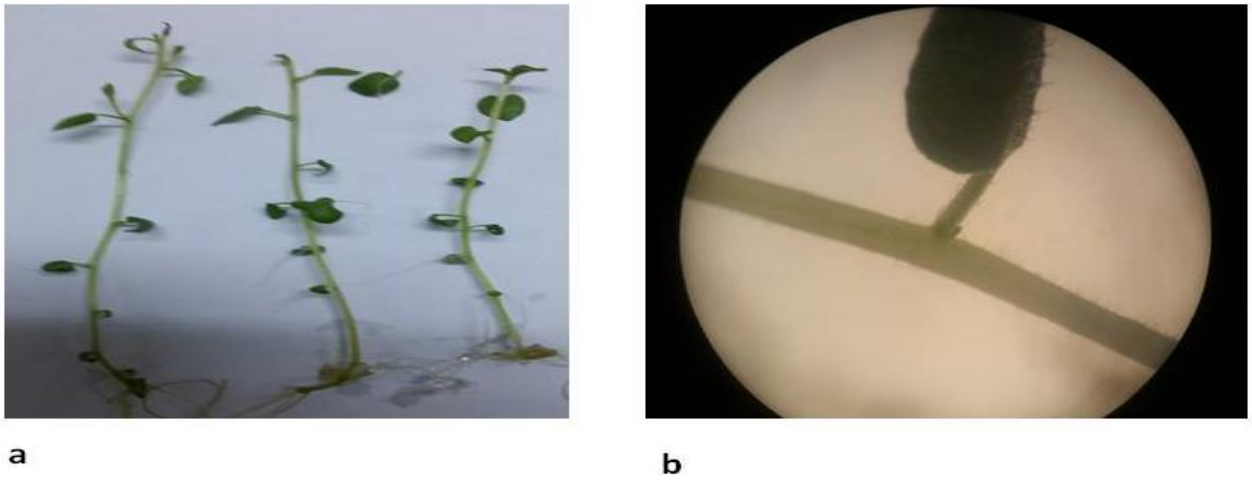

Fig. 1: (a) Leaf disc explants taken from plantlets of potato cultivars for callus induction under *in vitro* NaCl stress and (b) Potato leaf node (5×5 mm) used as explants under Binuclear microscope

***Ex vitro* experiment:** To examine the tuberization potential of the tested potato cultivars under salt stress, 5-week-old in vitro regenerated plantlets from the previous experiment, of cvs. Spunta, Nicola and Hermes were removed from the culture jars, washed with tap water to remove the excess of agar and soaked in fungicide solution (2.0 g L^{-1} Benlate) for 5 min, and transferred into a glasshouse. Plantlets were cultured in clay pots (25 cm) filled with peat moss and vermiculite (3:1 v/v), each pot included 3 plantlets. Salt and ZnO-NPs treatments were applied through irrigation with nutrient solution containing 2 g L^{-1} of (19-19-19 NPK) supplemented with NaCl at (0.0, 1.0, 2.0, 3.0 and 4.0 g L^{-1}) and ZnO-NPs at 0.0 and 50 mg L^{-1} twice a week starting from 01 January 2019 to 28 February 2019. Potato tubers were harvested on 30 March 2019. The plants were kept at 22/16°C (16 day/8 night) and undergone an average humidity of 65% for the whole cultivation phase. Data on tuber number and weight were recorded of at final harvest.

Chemical analysis of potato tubers: Samples of tuber were dried at 70°C in an oven then ground. Dry powdered samples (0.5 g) were digested by a wet digestion method following Jackson (1973). Total tuber nitrogen was determined by the Kjeldahl method (Chapman and Pratt, 1961). Crude protein was estimated by multiplying total nitrogen with a factor 6.25 (AOAC, 1995). Total calcium (Ca) and iron (Fe) concentration in tuber extracts were determined by an Inductively Coupled Plasma (ICP-OES, Spectro Analytical Instruments, Kleve, Germany) according to Sen and Pendam (2016).

Statistical analysis: Experimental data were analyzed by Fisher's analysis of variance using statistical software Costat (2011). Duncan's new multiple range test was used for mean separation (Duncan, 1955).

RESULTS

The analysis of variance showed that potato cultivars differed significantly ($p \leq 0.001$) for callus fresh (CFW) and dry (CDW) weights, tuber number, tuber weight, tuber protein, tuber iron and tuber calcium. Salt stress and application of ZnO-NPs also affected all the studied parameters significantly, except for tuber protein by ZnO-NPs application (Table 1). Two-way (cultivars × salinity and salinity × ZnO-NPs) and three-way (cultivars × salinity × ZnO-NPs) interactions were significant for all the parameters except for CDW and number of tubers

in case of three-way interaction. Two-way interaction of cultivars × ZnO-NPs was only significant for CFW, tuber protein, and tuber iron (Table 1).

Under *in vitro* culture conditions, embryogenic calli were successfully induced and proliferated on MS medium supplemented using varying plant growth regulators (Fig. 2). The maximum CFW was recorded in cv. Spunta (0.315 g) followed by cv. Hermes (0.152 g) and cv. Nicola (0.124 g) over their respective control. Comparing the results of different levels of salinity and ZnO-NPs on CFW showed that addition of NaCl salt to the growth medium led to a negative impact on the growth of callus, which caused a reduction in CFW and this effect is directly related with the concentration of NaCl salt in the medium (Fig. 2). The cultivar Spunta was the least affected by the salt stress followed by the cv. Hermis and cv. Nicola (Fig. 3). The application of ZnO-NPs significantly improved the growth of callus under salt stress and normal conditions. Highest CFW (0.491 ± 0.064a; 0.270 ± 0.058a; 0.378 ± 0.091 a) were recorded in cvs. Spunta, Nicola and Hermis with the application of ZnO-NPs under 17.0, 68.0, and 34.0 mM NaCl levels of salt stress, respectively (Fig. 3). Application of ZnO-NPs, under salt stress levels to 17 and 34 mM NaCl in the growth media, produced the highest CDW in cvs. Spunta (0.0351 g) and Hermes (0.0194 g), while the CDW was highest in cv. Nicola (0.0194 g) under control treatment without ZnO-NPs application (Fig. 3).

Table 1: Analysis of variance for the influence of salt concentration and ZnO-NPs on callus growth (g), tuber protein (%), tuber minerals (%) and yield components (g) of potato cultivars.

SOV	df	Mean Sum of Squares						
		CFW	CDW	Protein	Calcium	Iron	Tubers number	Tuber weight
Main effects								
Cultivars	2	0.1289***	5.670***	0.3574***	0.0124***	21.49***	69.03***	113.40***
Salinity	4	0.0358***	2.465*	0.0480***	0.0123***	223.54***	66.15***	48.304***
Zinc-NPs	1	0.1688***	4.199*	0.0033^{ns}	5.329***	13.814***	12.1**	102.122***
Interactions								
Cultivars × Salinity	8	0.0215***	2.336*	0.0284***	0.002***	9.040***	3.908*	19.92***
Cultivars × Zinc-NPs	2	0.0234**	2.500^{ns}	0.0128***	7.09^{ns}	0.898***	1.633^{ns}	2.097^{ns}
Salinity × Zinc-NPs	4	0.0161**	2.613*	0.0179***	2.87**	1.033***	5.405*	5.082***
Cultivars × Salinity × Zinc-NPs	8	0.0201***	1.692^{ns}	0.0064***	1.593**	0.587***	1.230^{ns}	11.21***
Error	68	0.0040	9.040	1.723	4.5722	0.0182	1.522	0.783
CV%		30.90	64.48	1.0098	1.971	1.898	17.056	5.250

*, **, ***Significant at p≤0.05, p≤0.01 and p≤0.001, respectively.
ns = Non-significant; SOV = Source of variance; df = Degrees of freedom; CV = Coefficient of variance; CFW = Callus fresh weight; CDW = Callus dry weight

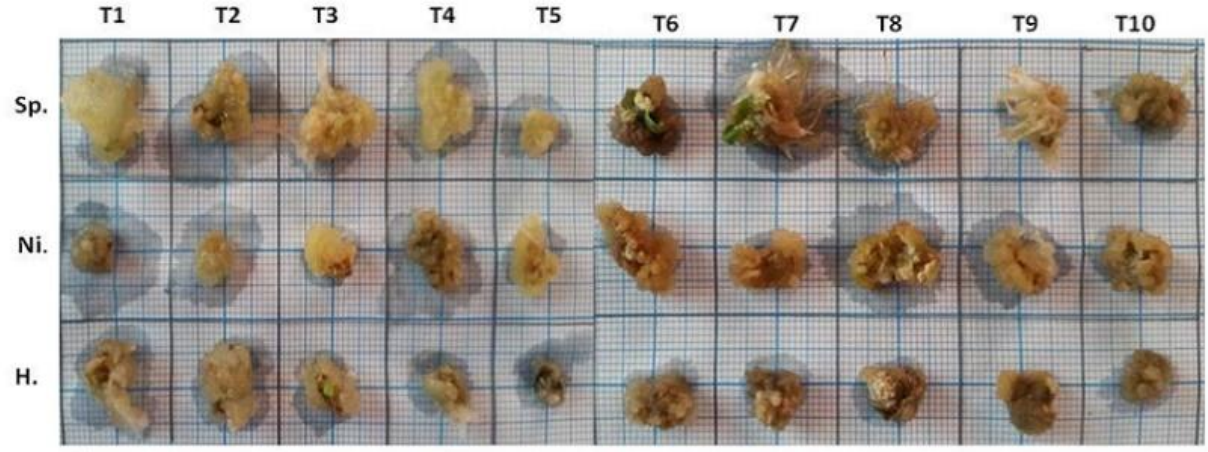

Fig. 2: Six-week-old embryogenic callus of potato cultivars Spunta (Sp.), Nicola (Ni.) and Hermis (H.) under different degrees of salt stress minus (T1-T5) or plus (T6-T10) addition of ZnO-NP to MS medium.

T1=0.0 mM NaCl; T2=17.00 mM NaCl; T3=34.00 mM NaCl; T4=51.00 mM NaCl; T5=68.00 mM NaCl; T6= 50 mg L^{-1} ZnO-NPs; T7= 17.00 mM NaCl+50 mg L^{-1} ZnO-NPs; T8= 34.00 mM NaCl +50 mg L^{-1} ZnO-NPs; T9= 51.00 mM NaCl+50 mg L^{-1} ZnO-NPs; T10= 68.00 mM NaCl +50 mg L^{-1} ZnO-NPs.

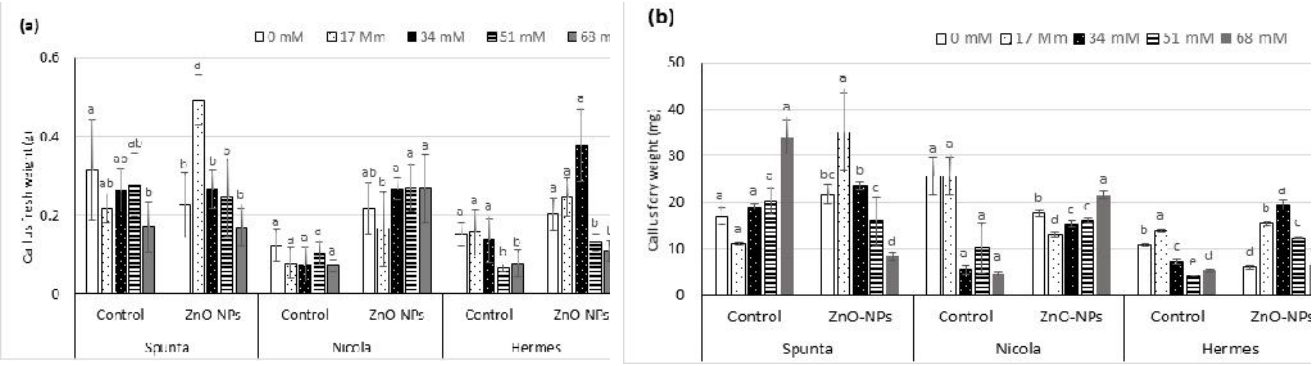

Fig. 3. Influence of ZnO-NPs on (a) callus fresh and (b) dry weight of potato cultivars under salt stress.
Data are represented as mean ± standard deviation from three replicates.
Means sharing the same letters, within each set of bars for a parameter, do not differ significant at $p \leq 0.05$.

Salt stress significantly reduced the tuber protein in potato cultivars. However, potato cultivars differ greatly for tuber protein content at different salt stress levels. The cultivars also respond differently to ZnO-NPs application. In cv. Spunta, lowest tuber protein was recorded at 34 mM NaCl stress for plants without ZnO-NPs application, whereas plants receiving ZnO-NPs application accumulated more tuber protein when grown at 0.0 or 34 mM NaCl. Nevertheless, the highest tuber protein contents were observed at 51 mM NaCl salinity stress with or without ZnO-NPs application (Table 2). In cv. Nicola, the highest tuber protein was recorded at 51 and 68 mM NaCl-induced salinity stress with and without ZnO-NPs application respectively, while plants grown under control or at 17 mM NaCl stress condition exhibited lowest tuber protein contents irrespective of ZnO-NPs application. In cv. Hermes, potato tuber harvested from plants grown at 34 mM NaCl with ZnO-NPs application accumulated the highest protein, while plants receiving ZnO-NPs accumulated more protein in tubers when grown at 17 and 34 mM NaCl. However, the lowest tuber protein was observed in plants grown under normal growth conditions irrespective of ZnO-NPs application (Table 2).

Salinity stress significantly decreased tuber calcium. However, the cultivars greatly differed for calcium (Ca) concentration and response to ZnO-NPs application. In the case of cv. Spunta, plants grown under control condition, and at 34 mM NaCl without ZnO-NPs application showed the highest tuber calcium accumulation, while plants the receiving ZnO-NPs accumulated more tuber calcium under control conditions. Furthermore, the highest salinity level (68 mM NaCl) caused the farthest decline in tuber calcium concentration irrespective of ZnO-NPs application (Table 2). In cv. Nicola plants grown at normal conditions and not receiving ZnO-NPs had more calcium, while the lowest tuber calcium was recorded in plants grown at mild salinity stress (17 mM NaCl). However, potato plants receiving ZnO-NPs application accumulated more calcium in tubers at 51 mM NaCl, while the lowest tuber calcium concentration was recorded at 34 mM NaCl salinity level. In cv. Hemes, the highest and lowest Ca concentration was recorded in plants grown under normal and extreme saline conditions (68 mM NaCl) irrespective of ZnO-NPs application (Table 2).

Salt stress drastically reduced the tuber iron concentration in all three cultivars. Cultivars also differed significantly for tuber iron concentration. However, ZnO-NPs application reduced the extent of decrease in tuber iron concentration in all cultivars. In cv. Spunta, the lowest tuber iron concentration was recorded for a plant grown at 68 mM NaCl salinity stress, whereas the highest tuber iron concentration was recorded in control plants irrespective of ZnO-NPs application. In cv. Nicola, the highest iron concentration was recorded in control plants receiving no ZnO-NPs; while those treated with ZnO-NPs accumulated more iron when grown at 17 mM NaCl. Moreover, plants of cv. Hermes treated with ZnO-NPs showed more iron accumulation under noon-saline conditions, while plant grown with ZnO-NPs supply had the highest tuber iron concentration at 17 mM NaCl. Nevertheless, lowest tuber iron concentration was recorded for plants grown at 68 mM NaCl salinity stress, irrespective of ZnO-NPs application (Table 2).

The highest number of tubers were recorded for all three tested potato cultivars receiving ZnO-NPs under non-saline conditions, whereas the lowest number of tubers was recorded in plants under high levels of salinity with or without ZnO-NPs (51 mM NaCl; 68 mM NaCl; 51 mM NaCl + ZnO-NPs; 68 mM NaCl +ZnO-NPs). Overall, the number of tubers responded negatively to increasing levels of NaCl treatment with or without ZnO-NPs application. All three potato cultivars grown under 34 mM NaCl, regardless of ZnO-NPs application, had a significantly higher number of tubers compared to other treatments (Table 4). A significant increase in tuber weight, of tested potato cultivars, was recorded under 51.00 mM NaCl, regardless of ZnO-NPs treatment.

However, the average tuber weight was highest under 34 mM NaCl in cv. Hermes (Table 3; Fig. 4).

Fig. 4. Influence of ZnO-NPs on tuber yield of potato cv. Hermis under salt stress T1=0.0 mM NaCl; T2=17.00 mM NaCl; T3=34.00 mM NaCl; T4=51.00 mM NaCl; T5=68.00 mM NaCl; T6= 50 mg L^{-1} ZnO-NPs; T7= 17.00 mM NaCl+50 mg L^{-1} ZnO-NPs; T8= 34.00 mM NaCl +50 mg L^{-1} ZnO-NPs; T9= 51.00 mM NaCl+50 mg L^{-1} ZnO-NPs; T10= 68.00 mM NaCl +50 mg L^{-1} ZnO-NPs.

Table 2: Influence of ZnO-NPs on tuber protein, calcium and iron in three potato cultivars under NaCl-induced salt stress in vivo.

Cultivars	**Spunta**		**Nicola**		**Hermes**	
ZnO-NPs / NaCl mM	0.0 mg L^{-1} ZnO-NPs	50 mg L^{-1} ZnO-NPs	0.0 mg L^{-1} ZnO-NPs	50 mg L^{-1} ZnO-NPs	0.0 mg L^{-1} ZnO-NPs	50 mg L^{-1} ZnO-NPs
			Tuber protein (%) per 0.5 g dw			
00.00	1.410±0.0007 ^{b}	1.391±0.008 ^{d}	1.142±0.001 ^{d}	1.140±0.001 ^{d}	1.126±0.001 ^{e}	1.159±0.006 ^{c}
17.00	1.415±0.0014 ^{b}	1.491±0.001 ^{c}	1.1469±0.001 ^{d}	1.149±0.001 ^{d}	1.136±0.0008 ^{d}	1.366±0.011 ^{a}
34.00	1.377±0.001 ^{c}	1.377±0.010 ^{d}	1.1822±0.001 ^{c}	1.207±0.006 ^{c}	1.356±0.0013 ^{a}	1.388±0.0037 ^{a}
51.00	1.514±0.010 ^{a}	1.521±0.009 ^{a}	1.4132±0.001 ^{a}	1.220±0.007 ^{b}	1.295±0.0043 ^{b}	1.233±0.0057 ^{b}
68.00	1.376±0.001 ^{c}	1.375±0.001 ^{d}	1.291±0.009 ^{b}	1.311±0.002 ^{a}	1.229±0.0095 ^{c}	1.260±0.0143 ^{b}
LSD($p \leq 0.05$)	0.0083	0.0133	0.0071	0.0097	0.0086	0.0543
			Tuber calcium (%) per 0.5 g dw			
00.00	0.372±0.002a	0.381±0.006a	0.353±0.006a	0.346±0.011b	0.416±0.005a	0.411±0.001a
17.00	0.346±0.006b	0.341±0.001c	0.290±0.005d	0.294±0.005c	0.314±0.004c	0.361±0.005d
34.00	0.363±0.007a	0.367±0.001b	0.303±0.005c	0.294±0.005d	0.362±0.003b	0.361±0.005c
51.00	0.337±0.007b	0.339±0.005c	0.342±0.001b	0.360±0.005a	0.369±0.018b	0.344±0.005b
68.00	0.308±0.008c	0.321±0.010d	0.308±0.008c	0.303±0.005cd	0.327±0.001c	0.327±0.004d
LSD($p \leq 0.05$)	0.0125	0.0129	0.0108	0.0120	0.0159	0.0084
			Tuber iron (ppm)			
00.00	9.85±0.045a	9.79±0.085a	11.326±0.094a	11.516±0.076b	10.503±0.240b	10.55±0.287a
17.00	9.683±0.104b	6.066±0.058b	10.93±0.065b	11.72±0.070a	10.55±0.0866a	9.426±0.087b
34.00	5.05±0.050c	6.066±0.058b	5.146±0.136c	6.486±0.070c	8.676±0.0862c	9.426±0.087b
51.00	3.856±0.055d	3.95±0.10c	2.526±0.176d	4.303±0.035d	6.306±0.325d	8.30±0.090c
68.00	2.836±0.060e	3.303±0.100d	2.04±0.062e	2.38±0.155e	1.666±0.090e	3.75±0.219d
LSD($p \leq 0.05$)	0.1209	0.1602	0.2102	0.1659	0.3514	0.3570

Data are represented as mean ± Standard deviation from three replicates.
Means sharing the same letters, within a column for a parameter, do not differ significant at $p \leq 0.05$

Table 3: Influence of ZnO-NPs on number of tubers and tuber weight of potato cultivars under salt stress in vivo.

Cultivars		**Spunta**		**Nicola**		**Hermes**	
ZnO-NPs	NaCl mM	0.0 mg L^{-1} ZnO-NPs	50 mg L^{-1} ZnO-NPs	0.0 mg L^{-1} ZnO-NPs	50 mg L^{-1} ZnO-NPs	0.0 mg L^{-1} ZnO-NPs	50 mg L^{-1} ZnO-NPs
				Number of tubers /plants			
00.00		12.33±0.577a	12.66±0.577a	8.00±1.00a	10.00±1.732a	8.00±2.645a	7.33±1.154ab
17.00		8.00±1.00bc	8.33±0.577b	7.33±0.577a	7.33±1.154b	5.33±1.154ab	5.66±0.577bc
34.00		9.33±0.577b	11.00±1.00a	7.66±1.527a	10.66±1.527a	4.66±0.577ab	8.00±1.00a

51.00	6.33±0.577d	6.66±0.577bc	6.00±1.00ab	6.33±0.577b	5.00±2.00ab	4.66±0.577c
68.00	7.00±1.00cd	5.66±2.5c	4.33±1.154b	5.00±1.00b	3.66±1.527b	4.66±1.527c
LSD($p \leq 0.05$)	1.409	2.348	1.992	2.301	3.151	1.878
			Tuber weight (g)			
00.00	17.33±0.702b	20.1±1.212b	16.53±0.680a	18.76±0.251b	16.53±0.472a	16.43±0.450b
17.00	18.7±0.20b	19.93±0.602b	14.85±0.259b	17.06±0.115c	11.9±0.30c	14.3±1.053c
34.00	16.7±0.80b	18.9±0.529b	14.03±0.550bc	16.9±1.044c	14.3±0.793b	18.4±0.321a
51.00	21.56±2.315a	25.7±2.06a	17.5±0.854a	20.47±0.296a	17.13±0.115a	12.4±1.250d
68.00	14.03±0.896c	15.86±0.709c	13.73±0.378c	16.00±1.389c	11.63±0.568c	17.53±0.351ab
LSD($p \leq 0.05$)	2.204	2.135	1.063	1.451	0.9204	1.433

Data are represented as mean ± Standard deviation from three replicates.
Means sharing the same letters, within a column for a parameter, do not differ significant at $p \leq 0.05$

DISCUSSION

This study demonstrated that salt stress caused a significant reduction in the growth of callus in vitro as well as potato tuber number, tuber weight, tuber protein, tuber calcium, and tuber iron in vivo. Potato cultivars also differed significantly in response to salinity. However, in the current study the application of ZnO-NPs helped mitigate the adverse effects of salt stress in potato cultivars. Salinity affects plant growth through osmotic stress and mineral ion toxicity (Ghosh *et al*., 2001; Munns and Tester 2008). An increase in salt concentration in the rhizosphere soil disturbs the osmotic balance resulting in 'physiological/secondary drought', which restricts plant water uptake (Farooq *et al*., 2017). In this study, salt stress reduced the fresh and dry weight of callus. However, application of ZnO-NPs enhanced the callus fresh and dry weight of potato cultivars as ZnO-NPs are absorbed by plants to a greater extent and have high bioavailability owing to smaller particle sizes and low water solubility (Prasad *et al*., 2012), which enhance the chlorophyll contents, photosynthesis, and biomass production (García-López *et al*., 2019; Noohpisheh *et al*., 2020). Application of ZnO-NPs improved the tuber protein content under salt stress condition. Adequate Zn modulates the enzymes involved in the protein biosynthesis and thus enhance the protein content (Zhang *et al*., 2017; Rehman *et al*., 2018 b). Tuber protein contents were higher in salt-stressed plants due to lower tuber weight. There was great genotypic variation among the potato cultivars regarding protein content as cv. Spunta had the highest protein content under normal and salt stress conditions, irrespective of ZnO-NPs application.

Salt stress reduced the calcium and iron concentrations in tubers of all cultivars as salinity causes nutritional imbalances owing to decreased nutrient uptake and transport to the shoot. In this regard, Mirvat *et al*., (2006) reported that salt stress caused significant Zn imbalances in plant cells, which disturbs the normal plant metabolism. The application of ZnO-NPs helped in improving the ionic homeostasis and enhanced the calcium and iron uptake (Hezareh *et al*., 2020). The ZnO-NPs application enhanced the Zn availability which helped in better root growth and nutrient uptake and translocation (Rehman *et al*., 2019). Moreover, the application of Zn improves the calcium accumulation in roots (Rehman *et al*., 2019) and edible plant parts (Rehman *et al*., 2018 d) as was observed in this study. Zinc application caused significant improvement in calcium and iron uptake under salt stress by improving the structural integrity of root cell membranes and reducing the excessive uptake of the Na^+ under salt stress (Aktas *et al*., 2006; Noohpisheh *et al*., 2020).

Salt stress drastically reduced the yield and yield contributing traits of potato cultivars. The application of ZnO-NPs substantially improved the number of tubers per plant and potato tuber weight. This improvement was possible due to the involvement of Zn in carbohydrate metabolism, IAA metabolism, and ribosomal function (Rehman *et al*., 2018a). Furthermore, an increase in tuber weight and number with ZnO-NPs was the possible outcome of improved photosynthesis, plant growth, and nutrient uptake (Rehman *et al*., 2018c).

The use of nanodevices and nanomaterials offers an attractive option of micronutrient application in plants (Usman *et al*., 2020) including Zn. Zinc as nanomaterial may help improve plant growth and nutrient acquisition, under less than optimum conditions, than the traditional approaches as cited by Reynolds (2002) in accordance with the present study. The use of ZnO NPs may also help cope with the salinity-induced oxidative stress (Sheykh *et al*., 2009).

Conclusion: Salt stress significantly reduced the potato growth and yield; however, tuber protein concentration was higher at a high salinity level. Application of ZnO-NPs proved effective in ameliorating the salinity induced harmful effect on yield and quality of potato. The tested potato cultivars differed significantly for their response to salt stress. In this regard, the cultivar Spunta performed better under normal and salt stress conditions.

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