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Research Article
Nano-Zinc Oxide and *Arbuscular mycorrhiza* Effects on Physiological and Biochemical Aspects of Wheat Cultivars under Saline Conditions

[1]Hala Mohamed Safwat El-Bassiouny, [1]Maha Mohamed-Shater Abdallah, [2]Magda Aly Mahmoud El-Enany and [1]Mervat Shamoon Sadak

[1]Department of Botany, Agricultural and Biological Research Division, National Research Centre, 33 El Bohouth Street, P.O. Box 12622, Dokki, Giza, Egypt
[2]Department of Genetics and Cytology, Genetic Engineering and Biotechnology Research Division, National Research Centre, 33 El Bohouth Street, P.O. Box 12622, Dokki, Giza, Egypt

Abstract

Background and Objective: Saline soils are restrictive factors to agriculture in arid and semi-arid regions, plant growth and productivity. Thus, it was important to consider how, nano-zinc oxide or bulk zinc oxide alleviates the oxidative salt stress in the presence of *Arbuscular mycorrhiza* (AM) fungi by two wheat cultivars (Sids 13 and Sakha 94). **Materials and Methods:** A field experiment was carried out during two winter successive seasons to study the beneficial role of nano-zinc oxide or bulk zinc oxide with different concentrations (5 and 10 mg L^{-1}) in enhancing growth, some biochemical and physiological of two wheat cultivars under saline soil. **Results:** Soaking both wheat cultivars with nano-zinc oxide or bulk zinc oxide in the presence of AM improved growth parameters. All treatments increased significantly photosynthetic pigments, IAA, phenols contents, organic antioxidant enzyme activities and significant decrease in lipid peroxidation. some changes are observed in protein patterns, so several proteins were disappear, but others were selectively improved and synthesis of the new groups of protein was formed, some of these responses were observed through the effect of nano-zinc oxide or bulk zinc oxide and AM. **Conclusion:** Nano-ZnO (10 mg L^{-1}) in the presence of AM was the most effective treatments on both cultivars. Results showed superiority of Sakha 94 cultivar in most growth parameters and biochemical aspects than Sids 13 cultivar.

Key words: ZnO nanoparticles, *Arbuscular mycorrhiza*, wheat cultivars, protein patterns, saline soil

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Corresponding Author: Hala Mohamed Safwat El-Bassiouny, Department of Botany, Agricultural and Biological Research Division, National Research Centre, 33 El Bohouth Street, P.O. Box 12622, Dokki, Giza, Egypt

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Soil salinity becomes a serious problem in both agricultural and natural soils. Saline soils are restrictive factors to agriculture in arid and semi-arid regions, plant growth and productivety[1]. Plants sowing in saline soil primarily exposed to osmotic stress and secondarily ion toxicity stress. Specific ion influences may cause direct toxicity or may affect plant nutritional balances[2].

A possible approach to enhance plant growth and productivity under salinity stress is the application of *arbuscular mycorrhiza* fungi (AMF). Several studies have established that AMF protected the host plants to promote the growth of plants under environmental stress[3]. The AMF is vital in prospective agriculture as it improves plant water relations, it increases mineral uptake, accumulation of the osmoregulators, enhances in photosynthetic rate and efficiency of used water. The AM mitigated the salt stress as the result of a biochemical and physiological influnces[4]. It can break various complex minerals and organic compounds in the soil and make them obtainable to their hosts[5]. The AMF can progress plants by enhancing the production of growth bioregulators, ameliorating osmotic adjustment under salinity stress[6].

Zinc is an important nutrient for plants that is absorbed in the form of divalent cations and has different physiological functions in plants. Zinc is participating in the metabolism of proteins, carbohydrates, nucleic acids, lipids, photosynthesis and biosynthesis[7,8]. This element is used as part of the structure of enzymes or reacts as regulator cofactors in a number of enzymes. Zinc is used in the building of at least four enzymes: carbonic anhydrase, Cu-Zn superoxide dismutase, alcohol dehydrogenase, RNA polymerase[9]. The ZnO shares in regulation of hormone metabolism: It modifies auxin levels through tryptophan biosynthesis and it is necessary for activation of numerous enzymes, like superoxide dismutase and dehydrogenases[10].

Nanoparticles (NPs) are microscopic particles with at least one dimension less than 1000 nm. Therefore, these particles are much attractive materials to use in biological system. Nano-particles react with plants inducing several morphological and physiological changes, depending on the properties of nanoparticles Ma *et al.*[11] and Khodakovskaya *et al.*[12] suggested that, the positive and negative influences on plant growth and development, depend on the composition, concentration, size and physical and chemical properties of NPs as well as plant species. Nano ZnO stimulated somatic embryogenesis, regenerating of plantlets, shooting and improved tolerance to abiotic stress by stimulating proline synthesis, super oxide dismutase, catalase and peroxidase activities[13]. The ZnO plays a vital role in physiological and anatomical responses, zinc oxide nanoparticles are mostly used in agricultural applications[14]. ZnO-NPs improve seed germination, plant growth and development employing various plant species[15]. However, the influence of nanoparticles on plant secondary metabolism is still unclear. The nanoparticles stimulated the induction of the reactive oxygen species has been observed consistently via plant species[16].

Biochemical markers have received more attention in recent years as the data reflect more really the genetic variability for they are the direct products of genes (SDS-protein and isozymes) are proving increasingly valuable in providing input for genetic differentiation and conservation discussions, where there is a basic need to assess some measures of genetic variability in and among cultivars[17].

The main objective of the present work was to research the effect of mycorrhiza amended to the soil and/or nano-zinc oxide or bulk zinc oxide on growth, some physiological and biochemical aspects on wheat plants (*Triticum aestivum* L.) (Sids 13 and Sakha 93) cultivars grown under saline soil.

MATERIALS AND METHODS

The field experiment was carried out at the experimental station Wadi El-Natrun district El-Beheira Governorate, Egypt, (This region is part of the Sahara Desert of North Africa represent arid or semi-arid region), during the two winter seasons of 2016/2017 and 2017/2018. Grains of wheat cultivars (Sakha 94 and Sids 13) were obtained from the Agricultural Research Centre, Ministry of Agriculture, Giza, Egypt. *Arbuscular mycorrhiza* fungus was obtained from the Microbiology Department, National Research Centre. Zinc oxide nanoparticles (NPs) or zinc oxide bulk (BPs) used in the present work were supplied from Sigma-Aldrich.

The soil texture of the experimental site was sandy. Soil analysis and chemical analysis of irrigation water were performed according to the method described by Chapman and Pratt[18] (Table 1, 2).

The experimental design was split-split plot design with 4 replicates. The two cultivars occupy the main plots, where AM occupied in sub plots and treatment of primed seeds of wheat (Sakha 94 and Sids 13) cultivar with ZnO (NPs) or ZnO (BPs) (0, 5 and 10 mg L^{-1}) for 12 h before sowing were allocated at random to sub-sub plots.

Table 1: Physical and chemical analysis of the experimental site soil in 2016/2017 and 2017/2018 seasons

Soil analysis	2016/2017	2017/2018
Physical properties		
Sand (%)	94.15	92.27
Silt (%)	4.35	5.20
Clay (%)	1.50	2.53
Texture class	Sandy loam	Sandy loam
Chemical properties		
$pH_{(1:1)}$	7.43	7.29
$Ec_{(1:1)}$ (dS m^{-1})	5.54	5.22
Organic matter (%)	0.51	0.62
Total $CaCo_3$ (%)	3.74	5.91
Available N (mg kg^{-1})	6.4	8.9
Available P (mg kg^{-1})	1.65	2.04
Available K (mg kg^{-1})	168	187
Irrigation system	Drip irrigation	Drip irrigation

Table 2: Chemical analysis of irrigation water

		EC		Ions concentration (meq L^{-1})						
Seasons	pH	dS m^{-1}	ppm	HCO_3^-	CL^-	SO_4^-	Ca^{++}	Mg^{++}	Na^+	K^+
2016/2017	7.7	4.1	2624	2.8	30.5	9.0	3.9	4.3	33.3	0.64
2017/2018	7.5	4.2	2688	3.2	29.1	7.9	5.3	4.6	32.5	0.55

Wheat (*Triticum aestivum* L.) seeds were sown at the end of November, 25 th in both season in rows, 4 m long, the distance between rows was 25 cm, plot area was 12 m (3.0 m in width and 4.0 m in length). The recommended agricultural practices of growing wheat seeds were applied, the seeding rate was [144 kg seeds/hectare (ha)]. Pre-sowing, 360 kg ha^{-1} of calcium super-phosphate (15.5% P_2O_5) was applied to the soil. Nitrogen was applied after emergence in the form of ammonium nitrate 33.5% at rate of 180 kg ha^{-1} was applied at 5 equal doses before the 1st, 2nd, 3rd, 4th and 5th irrigation. Potassium sulfate (48.52% K_2O) was added at 2 equal doses of 120 kg ha^{-1}, before the 1st and 3rd irrigations. Irrigation was carried out using the new sprinkler irrigation system where water was added every 5 days. Plant samples were taken after 75 days from sowing for measurements of growth characters (shoot length (cm), number of leaves/tiller, fresh and dry weight of plant (g)). Biochemical aspects measured were photosynthetic pigments (chlorophyll a, b and carotenoids), IAA, phenol, TSS, proline, free amino acids, lipid peroxidation, H_2O_2, antioxidant enzymes, protein electrophoretic patterns.

Chemical analysis: Photosynthetic pigments: Chlorophyll a, b and carotenoids were determined using a spectrophotometer (Shimadzu UV-1700, Tokyo, Japan) described by Lichtenthaler and Buschmann[19]. Indole acetic acid content were extracted and analysed by the method of Larsen *et al.*[20]. Phenolic content was measured as described by Danil and George[21]. Total soluble sugars were extracted by the method of Prud'homme *et al.*[22] and analyzed according to Yemm and Willis[23]. Free amino acids and proline were extracted according to the method described by Vartanian *et al.*[24]. Free amino acid was determined with the ninhydrin reagent method[25]. Proline was assayed according to the method described by Bates *et al.*[26]. Enzyme extracts were prepared according to method of Chen and Wang[27]. Catalase (CAT, EC 1.11.1.6) and super oxide dismutase (SOD, EC 1.12.1.1) activity was calculated by nitro-blue-tetrazolium reduction method Chen and Wang[27]. Peroxidase (POX, EC 1.11.1.7) activity was evaluated according to Kumar and Khan[28]. The level of membrane damage was estimated by measuring malondialdehyde (MDA) according to Predieri *et al.*[29].

Electrophoretic analysis of protein by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). The SDS-PAGE was done according to Laemmli[30] as modified by Studier[31]. Polypeptide maps, molecular protein markers, percentage of band intensity, molecular weight and mobility rate of each polypeptide were related to standard markers using gel protein analyzer version 3 (MEDIA CYBERNE TICE, USA).

Statistical analysis: The data were statistically analyzed on complete randomized design under split plot system using MSTAT-C[32] software. Means were compared by using least significant difference (LSD) at 5% level of probability.

RESULTS

Growth parameters: Data in Table 3 showed that, both wheat cultivars (Sids 13 and Sakha 94) cultivated in the soil amended with AM led to a significant increase in the growth parameters (plant height (cm), leaves No./tiller, fresh and dry weight of tiller/g) when compared with plants cultivated without AM.

Data obtained revealed that presoaking both wheat cultivars with nano-ZnO or bulk-ZnO stimulated the all growth parameters in the presence or absence of AM when compared with the corresponding controls. More pronounced increase in growth parameters was obtained with plants treated with nano-ZnO (10 mg L^{-1}) in soil amended with AM over all other treatments in both cultivars.

Photosynthetic pigments: Data in Fig. 1a-d showed that, the cultivation of both cultivars (Sids 13 and Sakha 94) in the presence of AM induced a significant enhance in photosynthetic pigments (chlorophyll a, b, carotenoid and total pigments contents) as compared with the corresponding treatments in absence of AM. Results revealed that, significant increase in all photosynthetic pigment contents in response to treatment with either nano-ZnO or bulk ZnO on both wheat cultivars, in the presence or absence of AM as compared with control plants. The highest enhances of photosynthetic pigments were gained through soaking application with 10 mg L^{-1} nano-ZnO (40 and 34%) followed by 10 mg L^{-1} bulk-ZnO (26 and 12%) and amended with AM in Sids and Sakha 94, respectively. It is also noticed that, the highest carotenoid contents was found at concentration of bulk ZnO (10 mg L^{-1}) in Sids 13 and nano-ZnO (5 mg L^{-1}) in Sakha 94 cultivar in the presence of AM (Fig. 1c). Also, data clearly demonstrates that, cultivar Sakha 94 was surpassed Sids 13 as it provided the highest amount of photosynthetic pigments.

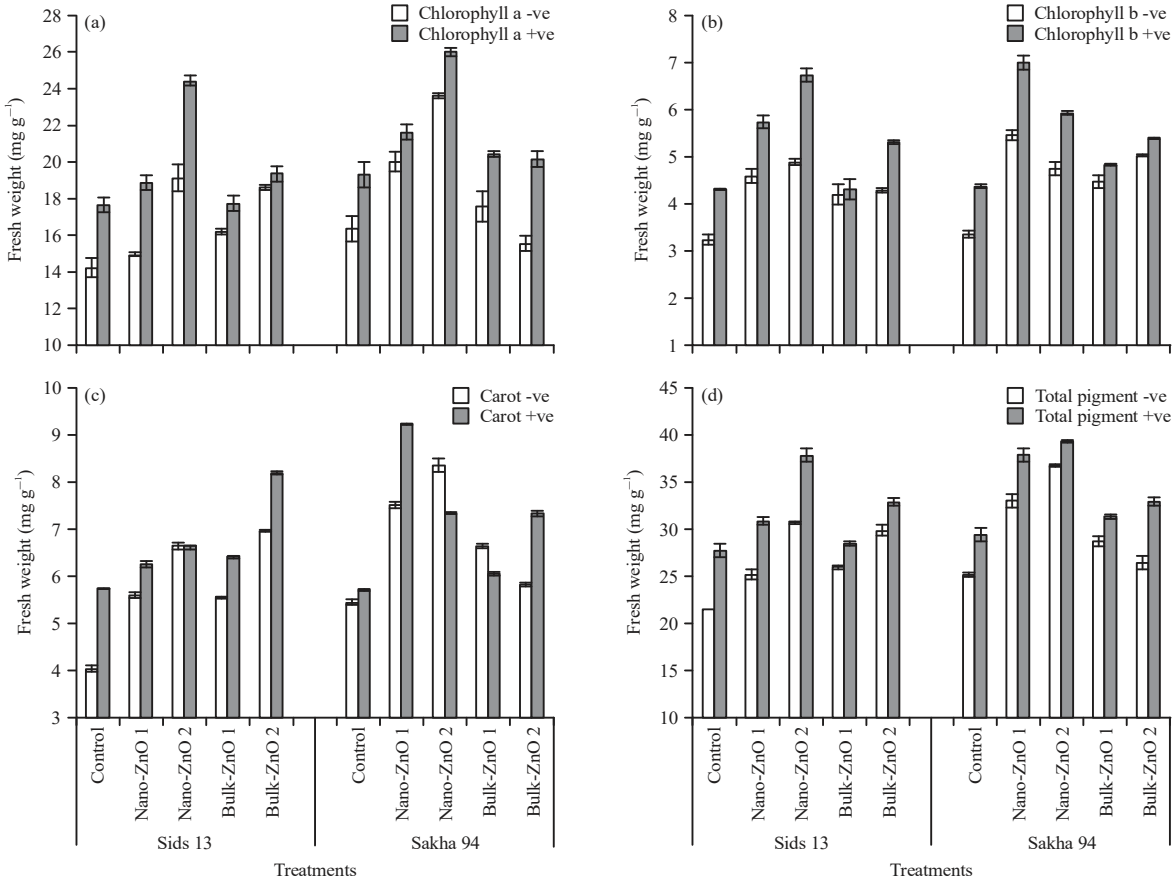


Fig. 1(a-d): Effect of nano-ZnO and bulk-ZnO (5 and 10 mg L^{-1}) in absence (-ve) or presence (+ve) of *Arbuscular mycorrhiza* on photosynthetic pigments, (a) Chlorophyll a, LSD 5% 1. 06, (b) Chlorophyll b, LSD 5% 0.17, (c) Carotenoids LSD 5% 0.33 and (d) Total pigments of wheat cultivars, LSD 5% 1.07 (Sids 13 and Sakha 94) in saline soil (at 75 days from sowing)
Each value represents the mean±standard error (n = 3)

Table 3: Effect of nano-ZnO and bulk-ZnO in absence (-ve) or presence (+ve) of *Arbuscular mycorrhiza* on growth of wheat cultivars in saline soil (at 75 days from sowing) (mean of two season)

		Plant height (cm)		Leaves No./tiller		Tiller fresh weight (g)		Tiller dry weight (g)	
Cultivars	Treatments	-ve	+ve	-ve	+ve	-ve	+ve	-ve	+ve
Sids 13	Control	43.90±0.33	48.58±0.34	6.13±0.02	6.37±0.02	3.32±0.01	3.59±0.04	1.15±0.01	1.29±0.06
	Nano-ZnO 5 mg L^{-1}	47.00±0.55	49.44±0.46	6.50±0.01	7.04±0.03	3.72±0.03	4.55±0.01	1.46±0.02	1.60±0.03
	Nano-ZnO 10 mg L^{-1}	48.67±0.33	51.71±0.54	7.23±0.01	7.67±0.03	4.14±0.01	4.73±0.05	1.51±0.04	1.74±0.05
	Bulk-ZnO 5 mg L^{-1}	49.09±0.62	51.53±0.13	6.28±0.04	6.77±0.03	4.45±0.05	4.59±0.07	1.60±0.06	1.58±0.02
	Bulk-ZnO 10 mg L^{-1}	48.76±0.84	50.50±1.15	6.58±0.02	7.18±0.08	4.22±0.02	4.43±0.04	1.45±0.02	1.70±0.04
Sakha 94	Control	49.00±0.41	50.33±0.13	6.18±0.04	6.34±0.06	3.61±0.04	3.67±0.02	1.24±0.03	1.31±0.03
	Nano-ZnO 5 mg L^{-1}	51.33±0.85	51.21±0.52	6.60±0.01	7.10±0.07	3.94±0.01	4.75±0.02	1.49±0.01	1.64±0.03
	Nano-ZnO 10 mg L^{-1}	51.93±0.082	56.50±2.33	6.19±0.02	7.67±0.09	4.78±0.03	4.81±0.03	1.67±0.03	1.80±0.04
	Bulk-ZnO 5 mg L^{-1}	51.67±0.71	57.33±1.45	6.95±0.03	7.07±0.06	4.41±0.02	4.72±0.01	1.49±0.02	1.64±0.02
	Bulk-ZnO 10 mg L^{-1}	52.22±0.43	53.56±1.22	5.96±0.01	7.08±0.08	4.48±0.03	4.60±0.03	1.49±0.03	1.71±0.01
LSD at 5%		2.72		0.53		0.22		0.16	

Each value represents the mean±standard error (n = 3)

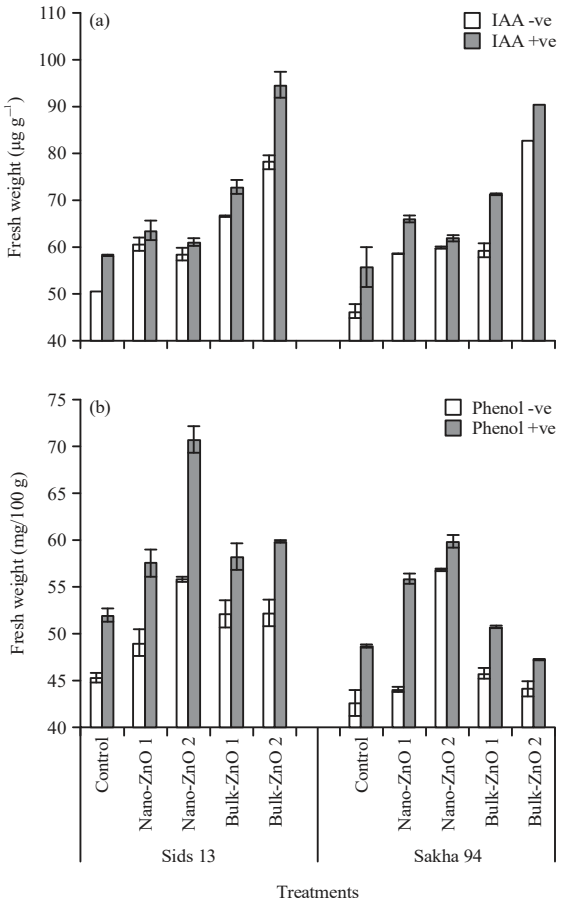


Fig. 2(a-b): Effect of nano-ZnO and bulk-ZnO (5 and 10 mg L^{-1}) in absence (-ve) or presence (+ve) of *Arbuscular mycorrhiza* on (a) IAA, LSD 5% 0.97 and (b) Total phenol contents of wheat cultivars, LSD 5% 1.49 (Sids 13 and Sakha 94) in saline soil (at 75 days from sowing)

Each value represents the mean±standard error (n = 3)

Change in IAA and phenol contents: Cultivation of both wheat cultivars (Sids 13 and Sakha 93) in the existence of AM led to significantly increases in indole acetic acid and phenolic contents as compared with the corresponding treatments in absence of AM (Fig. 2a, b). The percentage of increase in response to presence of AM reached to (15 and 16%) of IAA and total phenol in Sids cultivar and (20 and 14%) in Sakha cultivar, respectively as compared with untreated soil.

Results also revealed that, increases in IAA and phenol contents in response to treatment with either nano-ZnO or bulk-ZnO on both wheat cultivars, in the existence or absence of AM as compared with control plants. The greatest increase of the IAA was gained throught application with 10 mg L^{-1} bulk-ZnO and amended with AM by 86 and 95% in Sids and Sakha 94 cultivars, respectively (Fig. 2a). However, the maximum increase of the phenol contents was obtained by application with 10 mg L^{-1} nano-ZnO and amended with AM by 50 and 40% in Sids and Sakha 94 cultivars, respectively (Fig. 2b).

Organic solutes: The effects of nano-ZnO and bulk-ZnO on both wheat cultivars (Sids 13 and Sakha 93) cultivated in saline soil amended with AM, on some compatible solutes [total soluble sugars (TSS), proline and free amino acids, (FAA) are presented (Fig. 3a-c). The AM led to significant increases in total soluble sugars, proline and free amino acids of both wheat cultivars as compared with the corresponding treatments in absence of AM. The percentage of increase in response to presence of AM reached to (8 and 9%) of total soluble sugars, (28 and 33%) in proline and (28 and 33%) in total free amino acid in Sids 13 and Sakha 94 cultivars respectively as compared with untreated soil.

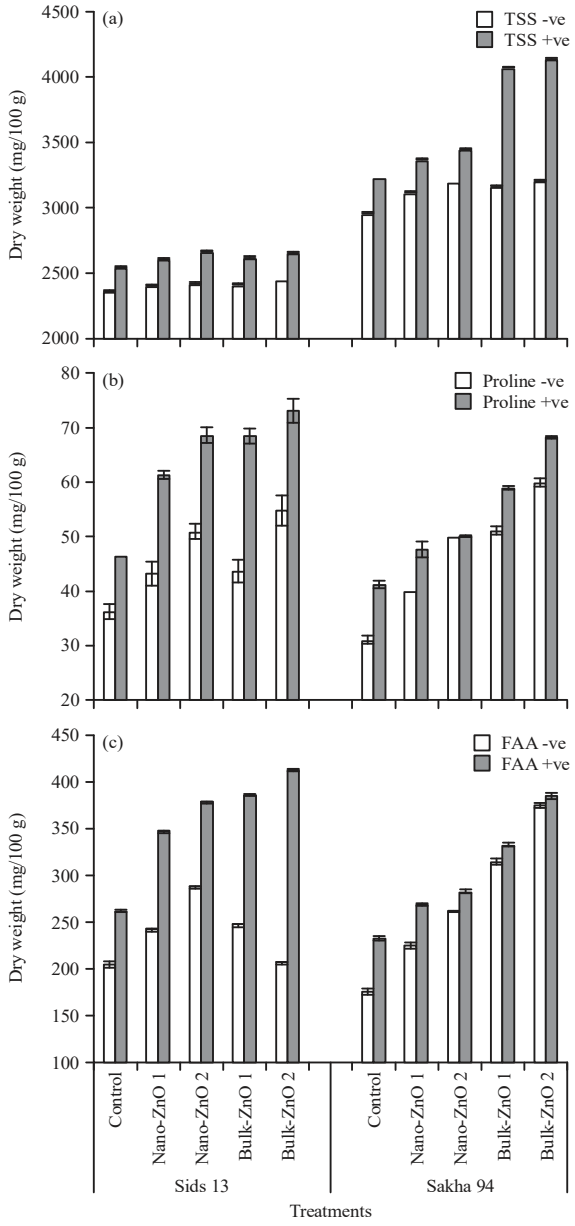


Fig. 3(a-c): Effect of nano-ZnO and bulk-ZnO (5 and 10 mg L^{-1}) in absence (-ve) or presence (+ve) of *Arbuscular mycorrhiza* on, (a) Total soluble sugar (TSS) LSD 5% 6.62, (b) Proline, LSD 5% 1.74 and (c) Total free amino acid (FAA), LSD 5% 10.07 of wheat cultivars (Sids 13 and Sakha 94) in saline soil (at 75 days from sowing)
Each value represents the mean ± standard error (n = 3)

Results also revealed that, increase in TSS, proline and FAA in response to treatment with either nano-ZnO or bulk-ZnO on both wheat cultivars, in the presence or absence of AM as compared with control plants. The maximum increase of the TSS was gain through application with 10 mg L^{-1} nano-ZnO and amended with AM in Sids 13 and 10 mg L^{-1} bulk-ZnO Sakha 94 cultivars (Fig. 3a). However, the maximum increases of the proline and free amino acid contents was obtained by application with 10 mg L^{-1} bulk-ZnO and amended with AM in both cultivars (Fig. 3b, c).

Lipid peroxidation: Data in Fig. 4a showed that AM decreased significantly the lipid peroxidation by (18%) in Sids 13 cultivar and (30%) in Sakha 94 cultivar as compared with the corresponding control without AM.

Application of nano-ZnO and bulk ZnO on wheat cultivars decreased significantly the lipid peroxidation. The effect of nano-ZnO and bulk ZnO in the presence of AM decreased the lipid peroxidation than in the absence of AM as compared with the corresponding treatment. However, the maximum decrease of the lipid peroxidation was obtained by application with 10 mg L^{-1} bulk-ZnO and amended with AM in both cultivars.

Antioxidant enzymes: The effect of nano-ZnO and bulk ZnO on wheat both wheat cultivars (Sids 13 and Sakha 94) cultivated in soil amended with AM in saline soil on antioxidant enzyme activities (POX, SOD and CAT) are showed in (Fig. 4b-d). Cultivation of both wheat cultivars in the presence of AM led to significant increases in POX, SOD and CAT activities as compared with the corresponding control without AM. Results also revealed that, significant increases in POX, SOD and CAT activities in response to treatment with either nano-ZnO or bulk ZnO in the presence or absence of AM as compared with the corresponding control in both cultivars. Meanwhile, it is noticed that, the treatment with 10 mg L^{-1} nano-ZnO in the presence of AM induced the reduction in catalase activity in both cultivars as compared with the corresponding controls (Fig. 4d). The maximum increase of the POX was obtained by soaking application with 10 mg L^{-1} bulk ZnO on both cultivars and amended with AM (Fig. 4b). However, the maximum increase of the SOD activity was obtained by application with 10 mg L^{-1} nano-ZnO in Sids 13 and 5 mg L^{-1} bulk-ZnO Sakha 94 cultivars and amended with AM (Fig. 4c). In response to CAT, the maximum increase was obtained by application with 5 mg L^{-1} nano-ZnO and amended with AM in both cultivars (Fig. 4d).

Protein banding patterns: The alters in protein electrophoretic patterns in the leaves of Sids 13 and Sakha 94 cultivars, grown under salty soil conditions in absence and presence of AM and treated with nano-zinc oxide or bulk zinc oxide are shown in Fig. 5 and Table 4. A total number of 11 bands were detected with molecular weights (MWs) ranging from 10-283 kDa, whereas, seven bands were

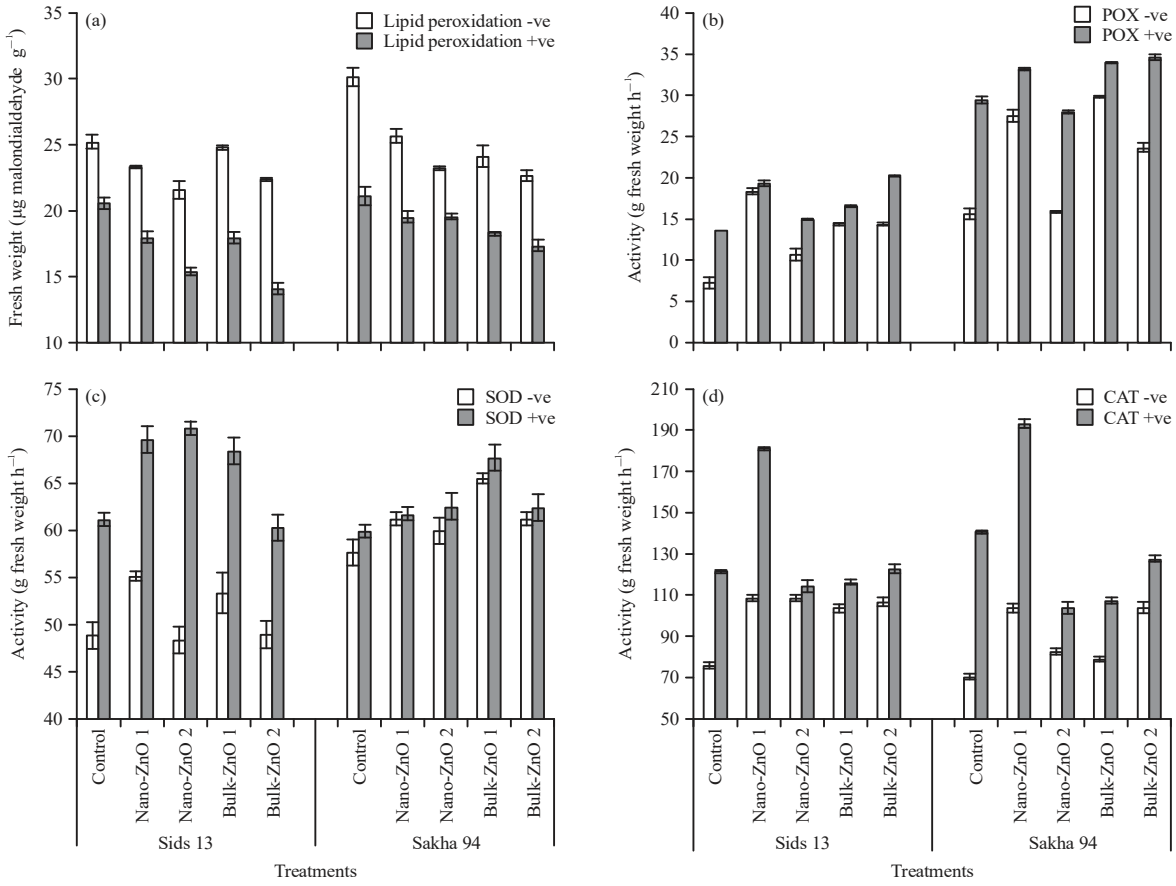
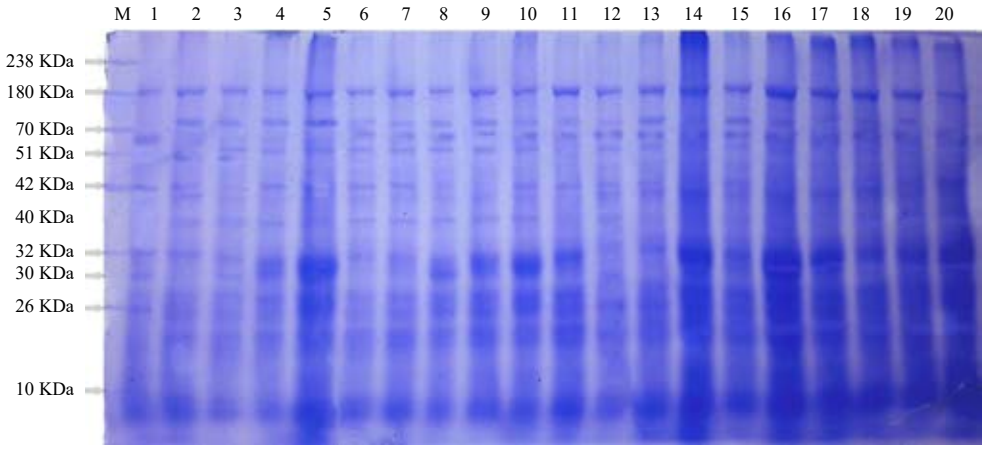


Fig. 4(a-d): Effect of nano-ZnO and bulk-ZnO (5 and 10 mg L^{-1}) in absence (-ve) or presence (+ve) of *Arbuscular mycorrhiza* on (a) Lipid peroxidation and enzyme activities, LSD 5% 1.67, (b) Peroxidase, LSD 5% 1.19, (c) Superoxide dismutase, LSD 5% 1.74 and (d) Catalase of wheat cultivars LSD 5% 1.65 (Sids 13 and Sakha 94) in saline soil (at 75 days from sowing)
Each value represents the mean±standard error (n = 3)

Table 4: Illustrates the analysis of electrographs of soluble protein patterns under a computer's program

		Sids 13										Sakha 94									
		-ve AM					+ve AM					-ve AM					+ve AM				
			n-ZnO		b-ZnO			n-ZnO		b-ZnO			n-ZnO		b-ZnO			n-ZnO		b-ZnO	
	Mwt	Con					Con					Con					Con				
Band No.	(KDa)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	180	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2	70		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
3	65	+					+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
4	51		+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+
5	42	+	+		+		+	+				+	+	+	+	+	+	+	+	+	+
6	40		+	+	+	+	+	+	+				+				+	+	+	+	+
7	32	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
8	30	+		+		+			+	+		+			+		+	+	+	+	+
9	26	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
10	20		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
11	10	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Number of bands		7	9	9	9	9	10	10	10	10	10	9	10	9	10	9	10	11	11	11	11

+: Presence of band, Mwt: Molecular weight, n-ZnO: Nano-ZnO, b-ZnO: Bulk-ZnO, Con: Control



Lane M: Marker

Sids 13				Sakha 94			
-ve AM		+ve AM		-ve AM		+ve AM	
Lane 1	Control	Lane 6	Control	Lane 11	Control	Lane 16	Control
Lane 2	Nano-ZnO 5 mg L^{-1}	Lane 7	Nano-ZnO 5 mg L^{-1}	Lane 12	Nano-ZnO 5 mg L^{-1}	Lane 17	Nano-ZnO 5 mg L^{-1}
Lane 3	Nano-ZnO 10 mg L^{-1}	Lane 8	Nano-ZnO 10 mg L^{-1}	Lane 13	Nano-ZnO 10 mg L^{-1}	Lane 18	Nano-ZnO 10 mg L^{-1}
Lane 4	Bulk-ZnO 5 mg L^{-1}	Lane 9	Bulk-ZnO 5 mg L^{-1}	Lane 14	Bulk-ZnO 5 mg L^{-1}	Lane 19	Bulk-ZnO 5 mg L^{-1}
Lane 5	Bulk-ZnO 10 mg L^{-1}	Lane 10	Bulk-ZnO 10 mg L^{-1}	Lane 15	Bulk-ZnO 10 mg L^{-1}	Lane 20	Bulk-ZnO 10 mg L^{-1}

Fig. 5: Electrographs produced by SDS-PAGE analysis of soluble protein patterns of wheat cultivars treatments with nano-ZnO or bulk-ZnO in absence (-ve) or presence (+ve) of *Arbuscular mycorrhiza* in saline soil (at 75 days from sowing)

polymorphic with 63.64%. With regard to protein banding patterns, four bands were monomorphic bands of 180, 32, 26 and 10 kDa with (36.36%) were recorded in all samples. The highest number of bands (11) were recorded in Sakha 94 (nano-ZnO 5 mg L^{-1}, nano-ZnO 10 mg L^{-1}, bulk-ZnO 5 mg L^{-1} and ZnO 10 mg L^{-1}) in the presence of AM, followed by 10 bands in control, nano-ZnO 5 mg L^{-1}, nano-ZnO 10 mg L^{-1}, bulk-ZnO 5 mg L^{-1} and bulk-ZnO 10 mg L^{-1} of Sids 13 and control Sakha 94 in the presence of AM and nano-ZnO 5 mg L^{-1}, bulk-ZnO 5 mg L^{-1} of Sakha 94 in the absence of AM. Moreover, it was observed variations in treated and untreated cultivars that expressed a protein bands with high intensity at the region of about 10-32 kDa in all cultivars except control gave low intensity of bands. However, the lowest number of polypeptides (seven polypeptides) was recorded in the control of Sids 13 cultivar in absence of AM. It is noted that absent two unique bands of 70 and 20 kDa in Sids 13 in control in absence of AM and 51 kDa in absence of AM in both cultivars. These bands could be considered as specific negative marker. However, the cultivar Sids 13 treated with *Arbuscular mycorrhiza* induced the induction the new protein bands. *Arbuscular mycorrhiza* stimulated the protein bands at Mwts (70, 51, 40 and 20 kDa) in Sids 13 cultivar and 51 and 40 kDa in Sakha 94 as compared to the corresponding control in absence of AM.

Zinc oxide-nano or zinc oxide bulk (5 and 10 mg L^{-1}) treatments increased the number of bands and density responsive proteins which were detected in absence and present of AM as compared to controls (Table 4). It is noticed that in cultivar Sids 13 treatments with nano-ZnO (5 and 10 mg L^{-1}) in the presence of AM exhibited ten bands comparing with nano-ZnO (5 and 10 mg L^{-1}) in the absence of AM exhibited nine bands with high intensity and density. In Saka 94 samples nano-ZnO (5 and 10 mg L^{-1}), bulk-ZnO (5 and 10 mg L^{-1}) in the presence of AM comparing with treatments in the absence of *Arbuscular mycorrhiza* exhibited variability of bands with high intensity and density.

DISCUSSION

Growth parameters: Cultivation of both wheat cultivars in the presence of AM led to significant increasing in growth parameters (Table 3). The application of AM improved plant growth and enhanced plant resistance to abiotic and biotic stresses[33-36]. These increases in the studied growth parameters can be resulted from the effects of AM on absorbing different nutriments like N, P, Ca, K, Cu, Zn and S[37].

The effect of nano-zino and bulk-ZnO stimulated the all growth parameters on Sids 13 and Sakha 93 cultivars (Table 3). The positive effect of ZnO on growth may be due to zinc is necessary for the synthesis of tryptophan which is a precursor of indole acetic acid production (Fig. 2) and consequently activating cell division, enlargement and regulation of plant growth[38,39]. Sedghi *et al*.[40] and Ramesh *et al*.[41] indicated that low concentration of ZnO-NPs induced useful effect on seed germination, increase plant growth and development in soybean and wheat plant, respectively.

The plants inoculated with AM led to a significant increase in photosynthetic pigments (Fig. 1a-d). Hajbagheri and Enteshari[42] indicated that *Arbuscular mycorrhiza* enhanced chlorophyll activity is reinstating because of presence of specific enzymes necessary for its biosynthesis. Borde *et al*.[43] observed that garlic plants inoculated with AM improved the photosynthesis rate under salt stress may be due to AM decreases Na level under salt stress.

Application of either nano-ZinO or bulk ZnO on both wheat cultivars, significant increase in photosynthetic pigment contents (Fig. 1a-d). Zinc has important in biochemical reactions needed for formation of chlorophyll and carbohydrates. Zinc is essential for the activity of enzymes that are participated in chlorophyll biosynthesis[44]. Raliya and Tarafdar[45], Munir *et al*.[46] and Ebrahimian and Bybordi[47] found that nano zinc oxide induced a significant improvement plant biomass, chlorophyll and protein synthesis in *Cyamopsistetra gonoloba* and wheat. Zinc protected sulfhydryl groups caused synthesized chlorophyll[48]. Generally, metallic nanoparticles are active effects of photosynthetic efficiency and this led to absorption of light by chlorophyll to increase[49]. Results also reveal that, significant increase in carotenoids content on both wheat cultivars (Fig. 1c). Carotenoids are antioxidant compounds which reduced oxidative damage of the plant[50]. Carotenoids are responsible protecting photosynthetic tissues, especially chlorophyll.

The AMF supplement to soil induced significant raises in IAA contents in both wheat cultivars (Fig. 2a). Liu *et al*.[51] and Abd Allah *et al*.[52] observed that, the inoculation of AM significantly enhanced IAA contents under stress conditions in corn and wheat plant. The plants inoculated with AM led to a significant increase in phenolic contents[53] in Cebil plant. It is possible to conclude that the application AMF results in various improves to the production of sets of phenolic compounds.

Application of either nano-ZinO or bulk ZnO on wheat cultivars, significant increase in, increase in IAA contents on both wheat cultivars (Fig. 2a). Zinc plays as an activator of enzymes in plants and is directly involved in the biosynthesis of auxin, which in turn indicates the increase in the growth of wheat plants[54]. Application of NPs is the main defense mechanism, as a possible initiator of oxidative stress of the plant via enhanced the secondary metabolism is mainly due to the increase in phenolic compounds (Fig. 2b) Doroteo[55]. García-López *et al*.[56], Raigond *et al*.[57] and Mohsenzadeh and Moosavian[58] observed that, nano-ZnO effect seedling growth and improved the accumulation of eligible phenolic compounds in different plants.

Data showed that, addition of AM increased TSS of both cultivars wheat plant (Fig. 3a). Abdallah *et al*.[36,52] and Porcel and Ruiz-Lozano[59] showed that, AM significantly enhanced TSS contents on sunflower, wheat and soyabean. Proline increased in both wheat cultivars as a result of mycorrhizal inoculation as shown in Fig. 3b). El-Bassiouny *et al*.[60] suggested that AM could enhance the osmotic adjustment of wheat plants through accumulation of proline. Proline acts as an osmoprotectant; it is also serves as a sink for energy to regulate redox potentials, as a hydroxyl radical scavenger[61]. Higher free amino acids in mycorrhizal plants relative to non-AM ones were attributed to enhanced resistance to salinity stress (Fig. 3c). Zhu *et al*.[62] showed that, mycorrhizal Zea *mays* L. had higher amino acids than non-mycorrhizal plants.

Results revealed an increase in total soluble sugars, proline and free amino acids in response to treatment with either nano-ZnO or bulk-ZnO on both wheat cultivars, in the presence or absence of AM (Fig. 3a-c). Abdel Latef *et al*.[63] reported that, seed-priming with Zn-NPs increased the levels of organic solutes (TSS, proline and FAA) in Lupine plant under salinity stress. Mohsenzadeh and Moosavian[58] showed that low concentrations of ZnO and ZnO-NPs significantly enhanced proline and soluble sugar in rosemary plant.

Figure 4a found that soil supplement with AM induced significant decreases in MDA contents in both wheat cultivars, which may be due to the AMF contributed to increase water absorption and nutrients uptake. Also, AM protected plants against oxidative stress by increasing antioxidant enzyme activities (POX, SOD and CAT) (Fig. 4b-d). The antioxidant enzymes have the ability to eliminate the ROS, as evidenced by decreasing accumulation of H_2O_2 protecting the plants against oxidative damage or stabilizing of membrane in turn enhancing salinity tolerance. Similar results were obtained by El-Bassiouny *et al*.[60] on sunflower plant.

Application nano-ZinO and bulk ZnO on both wheat cultivars induced significant decreased in the accumulation of MDA (Fig. 4a). This result in harmony with that obtained by Burman *et al*.[64], who found that Zn-NPs induced protective effects on membranes against changes of membrane

permeability and oxidative stress in chickpea plants. Moreover, Zinc is the important element acts as a co-factor for many enzymes that give to the appropriate performance of the antioxidant defense system. Zinc also stimulates the synthesis of metallothioneins, which are proteins effective in decreasing hydroxyl radicals and sequestration reactive oxygen species created in stress states[65].

Cultivation of both wheat cultivars in the existence of AM induced significant increases in POX, SOD and CAT activities in both cultivars (Fig. 4b-d). El-Bassiouny *et al.*[60] showed that, higher activities of SOD, CAT and POX, gave greater resistance to oxidative damage in mycorrhizal sunflower plants under water stress. Several researchers recommended that AM helps plants to improve salt stress by increasing the antioxidant enzyme activities could be due to the lower H_2O_2 concentration[66,67].

Results revealed that, significant increases in POX, SOD and CAT activities in response to treatment with nano-ZnO or bulk ZnO on two cultivars, in the presence or absence of AM. The higher antioxidant enzymes an activity was associated with lower accumulation of less lipid peroxidation (Fig. 4a). Abdel Latef *et al.*[63] and Faizan *et al.*[68] found that, the treatment of Lupine and tomato plant with ZnO-NPs significantly enhanced the antioxidant enzymes (CAT, POX and SOD). It is recognized that ZnO cooperates an important function in alleviating the stability of biomembranes and proteins through balancing the scavenging ROS production.

The cultivar Sids 13 supplemented with AMF induced the induction the new protein bands in leaves. These results were in agreement with those of Khalafallah and Abo-Ghalia[69] observed that, the soluble protein were higher in AM than non-AM plants during water stress and unstressed conditions. However, El Bassiouny *et al.*[60] found that, in the presence of AM led to the emergence of new proteins at Mwts (48, 40, 30 and 27 kDa) in sunflower plant. Wu and Xia[70] found that soluble protein in wheat plants increased in mycorrhizal plants. The AM can increase protein by delaying protein degradation and preserving normal metabolism of proteins.

Zinc Oxide-nano or zinc oxide bulk raised the number of bands and density responsive proteins in absence and present of AM (Fig. 5, Table 4). Dubchak *et al.*[71] demonstrated that, nano-particles obtained large surface to volume rate that promotes their bioavailability, bioactivity and biochemical activities. The protein bands at molecular weight 51 and 40 kDa in both cultivars can be considered as positive markers for ZnO-NPs and bulk ZnO and it was noted that these bands disappearing under the control treatment. In this connection, Abedi *et al.*[72] found that, in wheat plant the band with Mwt 51 kDa band might be related to Rubisco activase enzyme. Moreover, El–Bassiouny *et al.*[73] reported that protein with molecular weight of 40 kDa seems to dehydrin expressed under salinity stress in flax cultivars. This protein has a protective role in survival under salinity stress. In addition, Merrick and Bruno[74] and Thomas *et al.*[75] found that unique gene expression patterns may help in development and validation of promising biomarkers suitable for high-through put screening methods and for better understanding of the toxicity of nano-particles. Results show superiority of Sakha 94 cultivar in the number of protein bands and density responsive proteins than Sids 13 cultivar. In this connection, Ali *et al.*[76] reported that salt tolerance barley cultivar under salt treatment were recognize by a specific band and proposed that this specific bands might use as markers for the identification of tolerant cultivar under salt stress.

Application of zinc oxide nanoparticles and soil amended with Arbuscular mycorrhiza could be a sustainable and environmental safe treatment, inexpensive and ecofriendly. These substances improved the growth, physiological, biochemicals aspects and elevaited the deleterious effects of salinity stress in wheat plants.

CONCLUSION

The cultivation of both wheat cultivars in the presence of *Arbuscular mycorrhiza* fungi and either nano-zinc oxide or bulk zinc oxide increased growth parameters. They also increased various biochemical aspects (photosynthetic pigments, indole acetic acid, phenol, osmoprotectant contents and synthesis of the new group of responsive proteins). Moreover, these treatments caused a decreased in the lipid peroxidation and increased POX, SOD and CAT activities in both cultivars grown under saline soil. The interaction between cultivars and different concentrations of both type of ZnO showed that, 10 mg L^{-1} nano-ZnO in the presence of AM were the most effective treatments on both cultivars. Results show superiority of Sakha 94 cultivar in most growth criteria, biochemical aspects and the number of protein bands and density responsive proteins than Sids 13 cultivar.

SIGNIFICANCE STATEMENTS

The application of NPs and soil amended with Arbuscular mycorrhiza are the main defense mechanism, as a possible initiator of oxidative stress of the plant under salinity stress. These substances counteract the deleterious effect of salinity stress via increasing the secondary metabolism like phenolic compounds, antioxidant enzymes and number of responsive protein.

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