Plant Physiology and Biochemistry 207 (2024) 108383

Contents lists available at ScienceDirect

Plant Physiology and Biochemistry

journal homepage: www.elsevier.com/locate/plaphy

Magnesium nanoparticles extirpate salt stress in carrots (*Daucus carota* L.) through metabolomics regulations

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ARTICLE INFO

Handling Editor: Shivendra Sahi

Keywords:
Daucus carota L
Salt stress
Magnesium oxide nanoparticles

ABSTRACT

Underground vegetables are sensitive and vulnerable to salt stress. The vegetables are the main source of vitamins, nutrients and minerals in human diet. Also contain healthy carbohydrates, antioxidant and resistant starch which are beneficial for human health. Salinity influences water balance, morphological appearance and cellular interference of crop plants. It also caused disproportion of nutrients which usually affects the physiochemical processes in plant. Salt stress also affect biochemical attributes and hampers the growth of underground organs, due to which yield of crop decreased. The nanoparticles had been potentially used for better crop yield, in the recent. In our research study, we elaborate the positive response of magnesium oxide nanoparticles (MgO-NPs) on the morphological and biochemical parameters as well as anti-oxidant enzymes action on two accessions of carrot (*Daucus carota* L.) under salt stress of 40 mM and 80 mM. In a pilot experiment, various levels (0, 50, 100, 150, 200 and 250 mg/L) of MgO-NPs were tested through foliar application on carrot plants. Foliar application of MgO-NPs at concentration of 150 mg/L was most effective treatment and ameliorate the salt stress in both carrot accessions (DC-03 and DC-90). The MgO-NPs significantly enhanced the morphological and biochemical parameters. The yield was significantly increased with the exposure of MgO-NPs. Our results thus confirmed the potential of MgO-NPs to endorse the plant development and growth under salinity. However, further research study is needed to explore effectiveness of MgO-NPs in various other plants for the ameliorant of salinity.

1. Introduction

Carrot (*Daucus carota* L.) belongs to the family of Apiaceae and firstly cultivated in Mediterranean region. It contains riboflavin, carbohydrates, protein, fat, carotene and vitamin C. Carrot contains sucrose which is mostly present in carrot roots with sugar contents, it is 10 times greater than fructose and glucose. Carrot also contains many medicinal importance. It can improve the vision at night. Worldwide, high yield carrot producing countries included, Belgium (47.64 tons/ha), United Kingdom (44.28 tons/ha), China and India (30–38.54 tones/ha). Pakistan had very low per hectare yield (17.5 tons/ha) in comparison to World's leading countries (Tanveer et al., 2012).

Salinity means soil affected with a large amount of salts which are soluble such as sulphates and chlorides of sodium, magnesium and also calcium (Kiełkowska et al., 2019). The problem of salinization of soil is growing rapidly for agriculture worldwide and causes a serious threat to future food production and crop yields. At high concentration the salt accumulates in soil and caused the inhibition of plant growth by causing injuries to the cells in transpiring leaves and by reducing their capability to take up water (Smoleń et al., 2020; Tabassum et al., 2024). The first stage of growing plant is germination, which is badly affected by salt stress. The germination of yellow carrot which is sensitive to the salt stress, were badly affected at 150 mM NaCl because root and shoot length was decreased by 80% (Nasircilar et al.). The severity of damage due to salinity varies from crop to crop. It also causes reduction in plant growth that causes many problems in the potency of crop especially in

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https://doi.org/10.1016/j.plaphy.2024.108383
Received 19 November 2023; Received in revised form 25 December 2023; Accepted 16 January 2024
Available online 23 January 2024

the areas where salt affects the lands (Chatterjee and Majumder, 2010; Usman et al., 2023). The root growth is less affected at low concentration of salinity other than shoot growth. There are different management methods of salinity control in different crops such as: salt leaching, primer and companion plants, bio-fertilizers, manure, seed priming, compatible solutes and foliar application of nutrients (Devi and Arumugam, 2019).

Nanotechnology had gained the attention of the scientists and is thought to be beneficial and crucial in different fields such as industry, electronic, medicines, energy, agricultural and environment (Akhtar et al., 2013; M. Zhang et al., 2015). The application of nanoparticles in agriculture is to improve the productivity of crops by increasing the plant nutrition, water use efficiency, precision farming, innovative tools for pathogen detection and environmental protection (Panwar, 2012; Walker, 2005). Micronutrient can also play their roles in resistance to the severe effect of toxic ions. By spraying the zinc, iron and magnesium on the shoots of wheat can improve the characteristics of growth under salinity effect (Ahmad and Akhtar, 2019). Nanoparticles (NPs) like ZnO, Zn, Fe_3SO_4 and Al when applied to vegetables and crop plants (radish, lettuce, cucumber, grape and maize) quickly enhanced root growth. Silver, ZnO and Sulphur NPs improved seedlings growth and development when applied on wheat, mung bean, and tomato, respectively (Zahedi et al., 2020). NPs with larger specific surface area operate as particular agrochemical carriers. Hence, enable site-specific regulated nutrient distribution and increased crop safety. Nanotechnology boost up input quality and reduces nutrient loss.

Crop productivity is significantly impacted by various variables, including diseases and abiotic stresses. Farmers have relied heavily on pesticides and conventional fertilizers to reduce crop losses, which is detrimental to human health and the sustainability of the environment (Shenashen et al., 2017). A variety of metallic NP i.e., copper and silver efficiently reduced yield loss through mitigating stresses and improved nutrient use efficiency. Careful utilization of NPs in agriculture can boost crop production without disturbing environment (Gondal and Tayyiba, 2022). Inorganic NPs (ZnO, Cu, SiO_2, TiO_2, CaO, MgO, MnO, and Ag) proved to be very effective against abiotic stresses as well as work as antimicrobial agents, thus, protect plants from abiotic and biotic stresses (Servin et al., 2015). Mg-NPs ameliorated salinity stress and improved *D. carota* growth and productivity. NPs for their unique properties, resulted in selective and improved nutrient absorption through soil and even foliar application (Batsmanova et al., 2013). Effective and careful use of NPs can fill up the space of conventional fertilizers and pesticides. Role of Mg-NPs in mitigation of salinity stress is less explored. The magnesium oxide nanoparticles (MgO-NPs) are best known for their potential for crop development, productivity and growth. Magnesium is importantly required for the growth of plant (Chen and Fan, 2018; Sawicki et al., 2017). Earlier studies proved that MgO-NPs at the concentrations of 10, 25, 50, 100 mg/L and at 5 g/L enhanced the germination and growth of mung bean, whereas MgO-NPs at 0.5 mg/L in peanuts promoted the seedling growth, seed germination and chlorophyll accumulation (Anand et al., 2020; Jhansi et al., 2017; Rani et al., 2020). In green bean, the 50 ppm of foliar application of Mg-NPs improved the photosynthetic pigments and highest amount of biomass was generated. The pods yield of green bean was improved at 100 ppm of Mg nanoparticles (Salcido-Martínez et al., 2020).

None of the study in previous literature focused on Mg-NPs use in mitigation of salt stress. But, so for studied literature revealed that Mg-NPs can be potentially used for improving crop productivity especially in stress prone areas. This was hypothesized that Mg-NPs can ameliorated salinity stress and improved *D. carota* physio-biochemical attributes under stress and control condition. This study was planned to study; effectiveness of Mg-NPs in the mitigation of salt stress, role of Mg-NPs to induce stress markers and respective ameliorant responses in *D. carota* accessions DC-03 and DC-90 and to asses the effect of Mg-NPs on various biochemical attributes of *D. carota* under stress and non-stress conditions.

2. Methodology

2.1. Experimental layout

Two accessions of *D. carota* DC-03 and DC-90 were used for this research work. Seeds of these two accessions were taken from Ayub Agricultural Research Institute (AARI), Faisalabad. The research was conducted to analyze the ameliorating role of magnesium nanoparticles (MgO-NPs) against the harmful effects of salts in *D. carota*. Firstly, a pilot experiment was carried out to choose the best level for MgO-NPs foliar spray. For the purpose, various levels of MgO-NPs (0, 50, 100, 150, 200 and 250 mg/L) were tested on *D. carota* grown under salinity. MgO-NPs had no or low effect on growth of carrot plants at low concentrations (50 and 100 mg/L) while it had negative effects at high level (200 mg/L) as equated to control. Carrot plants had no growth or become dead after some time when treated with 250 mg/L MgO-NPs. MgO-NPs when applied at 150 mg/L concentration showed the most effective and positive results and significantly improved carrot plant growth under saline conditions. For this reason, 150 mg/L concentration of MgO-NPs was used for further experimentation and testing. Experiment was conducted in 36 pots, with two accessions in a complete randomized design (CRD) with 3 replicates. Before sowing, pots were filled with sand having a hole of 1 inch, which was covered by cotton fabric. Sowing was done on 3rd November 2022 in prewash sand filled pots. Same size seeds at uniform distance were sown, 1/2-inch deep in sand. Germination started after a week and thinning of seedling was done after 3 weeks of germination. Each pot had 4-5 seedlings after thinning.

2.2. Salt levels and treatment of MgO-NPs

Salt with 3 levels (0, 40 and 80 mM), used after 40 days of seed germination in Hoagland's solution by gradual increase in salt concentration (from 20 to 40 mM) until to final concentration 80 mM. MgO-NPs (150 mg/L) was applied on same day along with final stress application by foliar spray method.

2.3. Characteristics of magnesium-oxide nanoparticles

The MgO-NPs have a number of advantages (Imani and Safaei, 2019). The MgO-NPs widely used in a medicinal and industrial processes, including catalysis, lithium-ion batteries, bone generation, antimicrobial and antibacterial inhibition, uranium sorption, toxic waste treatment, inhibition of drug, hazardous waste treatment and cryoinjury. MgO-NPs aid to degrade a component called methyl violet dye and help to remove phosphorus from wastewater, that hampers the plant growth. MgO-NPs for their unique properties such as the capacity to operate as anti-biofilm agents, also has ability of self-cleaning that assist to breakdown dye that inhibited the growth of plant (Fernandes et al., 2020).

2.4. Analyzing growth attributes

One week after treatments, single plant of average size was uprooted as sample from each of 36 pots. After that, samples were washed carefully and wiped with soft fabric. Using sharp edge blade, the roots of each sample were separated. Growth parameters like; shoot fresh and dry weight, carrot fresh and dry weight, number of leaves, shoot length and carrot length using weighing balance and measuring scale.

2.5. Membrane stability

2.5.1. Relative membrane permeability (RMP)

Membrane permeability was determined according to (Yang et al., 1996) protocol. The youngest leaves, approximately equal size, taken from every pot and then cut into tiny pieces. In test tubes having distilled water (20 mL), the chopped leaves were taken. These tubes were vortex

with LSE Vortex Mixer for few seconds (about 5sec) and the initial, electric conductivity (EC_0) was determined with the help of Milwaukee MW805 MAX pH meter. After that, test tubes were placed for 24 h at 4 °C and then EC_1 was obtained. Following that, same samples having leaves were autoclaved in SH- AC-60M for 20 min at 120 °C to determined EC_2. % of Relative membrane permeability (RMP) was obtained with the help of following formula:

$$\boldsymbol{RMP}\ (\%) = [EC_1 – EC_0\ /\ EC_2 – EC_0] \times 100$$

2.5.2. Determination of hydrogen peroxide (H_2O_2)

H_2O_2 level in plant tissues appraised by using 0.1 g fresh leaves from each replicate, following the (Velikova et al., 2000). Collected samples were grounded with pre-chilled pestle and mortar in the 5 mL of trichloroacetic acid (0.1% TCA). Then this homogenate shifted to falcon tubes and centrifuged at 12,000 g for 15 min at 4 °C by using HERMLE Z 326 K Universal centrifuge. In a test tube, the supernatant was separate from residue. 500 μL of the supernatant added to potassium phosphate buffer with neutral pH (500 μL) and 1 M KI (1 mL). Read absorbance of this mixture at 390 nm with the help of UV/VIS spectrophotometer.

2.5.3. Malondialdehyde (MDA)

By following protocol (Cakmak and Horst, 1991), lipid peroxidation estimated with few modifications. 0.2 g fresh leaf samples from every pot were homogenize at 4 °C in 3 mL of 1.0% trichloroacetic acid (TCA). Centrifugation of conical tubes containing that homogenate, was done at 2000 g for 15 min. In a test tube, obtained supernatant (0.5 mL) added to 3 mL of 0.5% Thiobarbituric acid (TBA), prepared in 20% trichloroacetic acid (TCA). These prepared samples were incubated in a shaking water bath of (Model: HSW-1/06) at 95 °C for 50 min. In order to stop reaction by cooling, the test tubes which contained reaction samples placed in an ice water bath. When the reaction stopped, these samples were centrifuge at 10,000×*g* for 10 min. Observed the absorbance of supernatant at wavelength of 532 nm using UV/VIS spectrophotometer. The thiobarbituric acid-reactive substances (TBARS) concentration was calculated, by using the absorption coefficient 155 $mmol^{-1}\ cm^{-1}$.

$$\boldsymbol{MDA\ level}\ (\boldsymbol{nmol}) = [(A_{532nm} – A_{600nm})/1.56 \times 10^5] \times V/W \times 10^6$$

2.6. Biochemical parameters

2.6.1. Proline content

According to (Bates et al., 1973) protocol, proline content was estimated. Fresh leaf (0.2 g) sample was homogenized in 3% of sulfo-salicylic acid (5 mL). Whatman filter paper was used to filter the homogenate. In test tubes, filtrate (2 mL) was taken along with acid ninhydrin (2 mL) and glacial acetic acid (2 mL). At 100 °C, this reaction solution was incubated for 60 min in an oven. The reaction was stopped by cooling in an ice bath by. The resultant solution was added to 4 mL toluene and mixed for 1–2 min. Chromophore (containing toluene) separated from the aqueous phase of mixture. UV/VIS spectrophotometer was used for reading absorbance at 520 nm against toluene (blank).

2.6.2. Extract preparation for estimation of antioxidant enzymes and total soluble protein

Fresh leaves (0.5 g) were homogenized in 10 mL of cooled 50 mM potassium phosphate buffer (pH 7.8). This homogenate was centrifuge for 20 min at 6000 g and 4 °C. For determination of total soluble proteins and antioxidant enzymes activities, the supernatant extracted from residue and then kept in deep freezer.

2.6.3. Total soluble protein content

Bradford solution was prepared as follows: 95% ethanol (50 mL) used to dissolve 0.1 g Coomassie Brilliant Blue. After that, added 100 mL of O-phosphoric acid (85%) and made the volume up to 1 L with distilled water. 0.1 mL of prepared extract (see previous section) mixed with 5 mL Bradford solution and then content was vortexed. UV/VIS spectrophotometer was used for reading the absorbance at specific wavelength (595 nm). Dilutions of bovine serum albumin were prepared and used to make standard curve of bovine serum. This curve used for the determination of protein in unknown samples (Bradford, 1976).

2.6.4. Activity of peroxidase (POD)

By following (Chance and Maehly, 1955) method, activities of POD enzymes estimated with minor changes. The POD reaction solution of 2 mL comprised of 600 μL of 20 mM guaiacol, 600 μL of 40 mM H_2O_2, 700 μL of 50 mM phosphate buffer (pH 5.0), and 100 μL enzyme extract. Thorough UV/VIS spectrophotometer, changes were recorded in the absorbance at 470 nm along within time limit of 150 s in steps of 30 s.

2.6.5. Superoxide dismutase (SOD) activity

Protocol of (Giannopolitis and Ries, 1977) was used for the determination of Superoxide dismutase activity, with some minor changes. SOD activity assessed by using potential of SOD antioxidant to prevent photoreduction of nitro-blue tetrazolium (NBT). The concentration of enzyme that prevented (50%) NBT photoreduction described one unit of SOD activity. Reaction solution (2.725 mL) contained 1.3 μM riboflavin, 50 μM NBT, 75 nM EDTA, 13 mM methionine and 50 mM phosphate buffer. 0.025 mL of enzyme extract and 0.25 mL of dis. H_2O added in the reaction solution to make total volume 3 mL. This reaction mixture was placed under light of 60 W for 20 min. UV–Visible spectrophotometer was used to read solution absorbance at 560 nm along with light and blank controls.

2.6.6. Total phenolic

Approximately equal size leaves from each replicate dried overnight in oven (80 °C). 0.25 mg dry grounded leaf sample were homogenized for 1 min in 5 mL of acetone (80%). Then homogenate was centrifuge at 3000 g for 15 min and supernatant of each extract taken to dryness. After that, added 5 mL of methanol in the left-over residue and mixed firmly for 1–2 min. Then methanol liquid layer separated from residue. In each sample, the amount of total phenolic was quantified by following Folin-Ciocalteu method. The reaction mixture in test tubes contained 1 mL of Folin-Ciocalteu's reagent, 2 mL of sample and 0.8 mL of sodium carbonate (7.5%). Then tubes were mixed and let it stand for 30 min. After 30 min, absorbance read by UV/VIS spectrophotometer at 765 nm. Content of Phenolic was determined as gallic acid equivalents (GAE) (mg) per dry material (g) (Kamath et al., 2015).

2.6.7. Inorganic ions

In test tubes, 0.1 g dried root and shoot powder was added in 2 mL H_2SO_4 and left for 24 h. The resultant mixture was heated on hot plate (Model: 85-2) in digestion flask and in the interim added H_2O_2 until mixture become colorless. After digestion, the mixture was filtered and diluted to make total volume 50 mL using distilled water (Wolf, 1982). Using Flame Photometer (Sherwood Model 360), cations (K^+ and Na^+) were estimated in prepared samples.

2.6.8. Yield parameters

Carrot length, carrot fresh weight and seed count were mounted after 3 months of experiment.

2.6.9. Statistical analysis

By following the Cohort software, 1988–2004 (CoStat statics program of version 6.303), ANOVA was applied on all parameters and LSD mean compare test was done with $p < 0.05$. MS excel was used to make graphs.

3. Results

3.1. Impression of MgO-NPs supplementation on growth parameter of D. carota under salt stress

3.1.1. Shoot fresh weight (SFW)

In accession DC-03 (Fig. 1-A), mild salt stress (40 mM) slightly decreased shoot fresh weight (10%), but SFW was effectively decreased by 43% under high salt stress (80 mM) as compared to control. In accession DC-90 (Fig. 1-A), the SFW was reduced by 14% and 40% under salt stress of 40 mM and 80 mM, respectively, in contrast to control. In accession DC-03, MgO-NPs foliar treatment increased SFW by 10% in plants encountering 40 mM salt stress. Whereas MgO-NPs foliar treatment increased SFW by 19% at 80 mM salt stress, as compared to untreated stressed plants. SFW was increased by 5% and 13% in accession DC-90 when treated with MgO-NPs under salt stress of 40 mM and 80 mM, respectively, as compared to untreated stressed plants. MgO-NPs significantly affected the SFW of carrot under salt stress. The interaction of all three factors Accessions * Salt Stress * Mg was non-significant regarding SFW (Table 1).

3.1.2. Shoot dry weight (SDW)

MgO-NPs significantly affected the SDW of carrot under salt stress as shown in Table 1. The mild stress (40 mM) decreased the SDW by 22% and high salt stress (80 mM) decreased SDW by 39% accession DC 03, as compared to control. In accession DC-90, the dry weight was reduced by 5% and 17% under salt stress of 40 mM and 80 mM, respectively, in contrast to control. In accession DC-03 (Fig. 1-B), MgO-NPs foliar treatment increased SDW by 17% in plants encountering 40 mM salt stress. Whereas MgO-NPs foliar treatment increased SDW by 26% at 80 mM salt stress, compared to untreated stressed plants. SDW was increased by 10% and 6% in accession DC-90 (Fig. 1-B) when treated with MgO-NPs under salt stress of 40 mM and 80 mM, respectively, as compared to untreated plants. The interaction of all three factors Accessions * Salt Stress * Mg was non-significant regarding SDW (Table 1).

3.1.3. Root fresh weight (RFW)

The interaction of all three factors Accessions * Salt Stress * Mg was non-significant (Table 1). With respect to RFW, remarkable difference was seen in both accessions of *D. carota*. Mild salt stress (40 mM) increased the RFW by 1%, whereas RFW was decreased by 28% under high salt stress (80 mM), in accession DC-03 (Fig. 1-C) as compared to control. However, in accession DC-90, the RFW was increased by 3% under mild stress (40 mM). and decreased by11% under high (80 mM) salt stress in accession DC-90, compared to control. The foliar application of MgO-NPs increased the RFW by 5% and 15% under salt stress of 40 mM and 80 mM, respectively, in accession DC-03 as compared to untreated stressed plants. Similarly, in accession DC-90 the RFW was increased by 13% and 20% by the application of MgO-NPs under 40 and 80 mM salt stress, as compared to untreated plants. Table 1 shows that the effect of salt stress and Mg nanoparticle on RFW was highly significant.

3.1.4. Root dry weight (RDW)

The interaction of all three factors Accessions * Salt Stress * Mg was non-significant regarding RDW (Table 1) In accession DC-03, the mild stress (40 mM) reduced the RDW (10%), also under high salt stress (80 mM), the RDW was decreased by 41% as compared to control. In accession DC-90, the RDW was reduced by 6% and 27% under salt stress of 40 mM and 80 mM, respectively, in contrast to control. In accession DC-03, MgO-NPs foliar treatment increased RDW by 7% in plants encountering 40 mM salt stress. However, MgO-NPs foliar treatment increased RDW by 50% at 80 mM salt stress, as compared to untreated stressed plants. RDW was increased by 1% in accession DC-90 when treated with MgO-NPs under salt stress of 40 mM. Conversely, under 80 mM salt stress, the MgO-NPs decreased (2%) the RDW, as compared to untreated stressed plants. MgO-NPs significantly affected the RDW under salt stress (Fig. 1-D).

3.1.5. Number of leaves

The mild (40 mM) and high (80 mM) salt stress decreased the number of leaves by 4% and 35%, respectively, as compared to control, in accession DC-03. In accession DC-90, the number of leaves was reduced by 9% and 14% under salt stress of 40 mM and 80 mM, respectively, in contrast to control. The interaction of all three factors Accessions * Salt Stress * Mg was non-significant regarding number of leaves (Table 1). In accession DC-03, MgO-NPs foliar treatment increased number of leaves by 4% in plants encountering 40 mM salt stress. Whereas MgO-NPs foliar treatment increased number of leaves by 12% at 80 mM salt stress, as compared to untreated stressed plants. The number of leaves were increased by 15% and 16% in accession DC-90 when treated with MgO-NPs under salt stress of 40 mM and 80 mM,

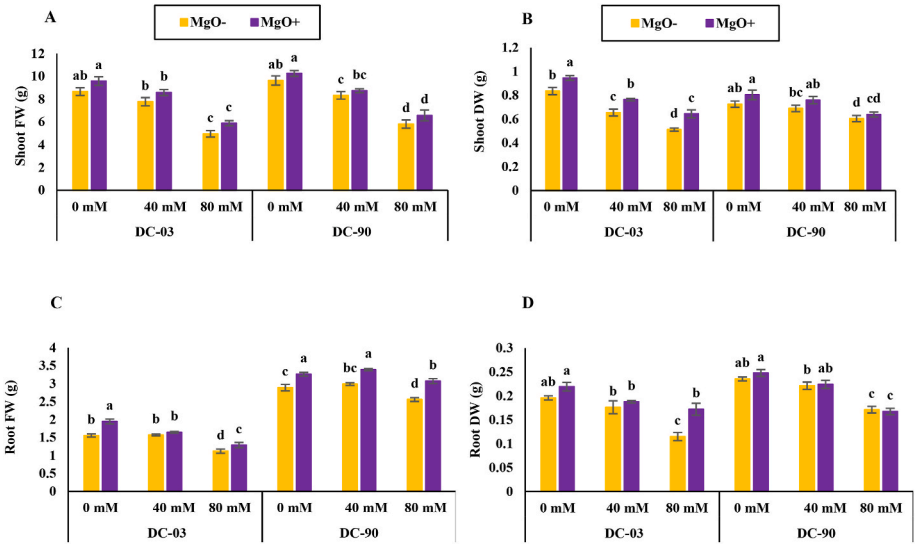

Fig. 1. Impression of MgO-NPs supplementation on shoot and root fresh weight and dry weight of D. carota accessions DC-03 and DC-90 under different levels of salt stress (MgO- represents untreated plants, MgO + represents plants treated with 150 mgL^{-1} MgO nanoparticles). Graph bars with same letters represent non-significant difference at $P \leq 0.05$.

Table 1
ANOVA for various *D. carota* attributes studied under different levels of salt stress with/without foliar supplementation of MgO nanoparticles.

Source of Variance	df	SFW		SDW		RFW		RDW		NL		SL		RL	
MS	P	MS	P	MS	P	MS	P	MS	P	MS	P	MS	P		
Accessions	1	3.80250 **	.0022	0.00453 ^{ns}	.1601	20.37620***	.0000	0.01014 ***	.0000	4.00000 **	.0020	44.44444 ***	.0000	24.667778 ***	.0000
Salt stress	2	43.56066 ***	.0000	0.15571 ***	.0000	0.62929 ***	.0000	0.01453 ***	.0000	11.08333 ***	.0000	60.53583 ***	.0000	0.8544444 *	.0480
Mg	1	4.92692 ***	.0007	0.07118 ***	.0000	0.91968 ***	.0000	0.00270 **	.0012	4.00000 **	.0020	18.20444 ***	.0000	1.6044444 *	.0177
Accession * Salt stress	2	0.19245 ^{ns}	.5599	0.02473 ***	.0003	0.07488 **	.0020	0.00017 ^{ns}	.4437	5.08333 ***	.0001	26.00361 ***	.0000	1.6677778 **	.0047
Accession * Mg	1	0.19331 ^{ns}	.4473	0.00710 ^{ns}	.0820	0.10693 **	.0023	0.00163 **	.0090	0.11111 ^{ns}	.5691	0.81000 ^{ns}	.2941	2.6677778 **	.0031
Salt stress * Mg	2	0.04518 ^{ns}	.8705	0.00009 ^{ns}	.9596	0.01652 ^{ns}	.1886	0.00029 ^{ns}	.2626	0.08333 ^{ns}	.7808	0.66361 ^{ns}	.4035	3.4411111 ***	.0001
Accession * Salt stress * Mg	2	0.00887 ^{ns}	.9730	0.00100 ^{ns}	.6348	0.03131 ^{ns}	.0505	0.00066 ^{ns}	.0564	0.19444 ^{ns}	.5658	0.58583 ^{ns}	.4472	1.1544444 *	.0194
Error	24	0.32389<-		0.00216<-		0.00923<-		0.00020<-		0.33333<-		0.70389<-		0.2472222<-	
Source of Variance	**df**	**RMP**		**H_2O_2**		**MDA**		**TSP**		**LP**		**TP**		**R Na^+**	
MS	P	MS	P	MS	P	MS	P	MS	P	MS	P	MS	P		
Accessions	1	7.17011 ***	.0001	27.18753 ***	.0001	0.22727 ^{ns}	.3012	0.00039 ^{ns}	.1930	0.00688 ***	.0000	76.61501 ***	.0000	173.36111 ***	.0000
Salt stress	2	114.94235***	.0000	131.96347 ***	.0000	3.75192 ***	.0000	0.00440***	.0000	0.00232 ***	.0000	15.65351 **	.0098	339.36111 ***	.0000
Mg	1	55.28399 ***	.0000	210.85460 ***	.0000	4.00333 ***	.0002	0.00054 ^{ns}	.1274	0.00911 ***	.0000	3.43114 ^{ns}	.2771	49.00000 ***	.0000
Accession * Salt stress	2	46.75498 ***	.0000	13.08514 ***	.0007	0.09044 ^{ns}	.6464	0.01019 ***	.0000	0.00448 ***	.0000	4.49423 ^{ns}	.2188	50.36111 ***	.0000
Accession * Mg	1	13.41801 ***	.0000	13.74556 **	.0034	0.05586 ^{ns}	.6052	0.00030 ^{ns}	.2524	0.00140 ***	.0000	0.04623 ^{ns}	.8984	1.36111 ^{ns}	.1764
Salt stress * Mg	2	4.91621 ***	.0001	21.28132 ***	.0000	0.01150 ^{ns}	.9452	0.00013 ^{ns}	.5607	0.00117 ***	.0000	27.00325 ***	.0008	2.33333 ^{ns}	.0531
Accession * Salt stress * Mg	2	8.99405 ***	.0000	2.52363 ^{ns}	.1659	0.04904 ^{ns}	.7878	0.00058 ^{ns}	.0878	0.00440 ***	.0000	17.04833 **	.0070	2.69444 *	.0357
Error	24	0.34332<-		1.30228<-		0.20353<-		0.00022<-		0.00005<-		2.77453<-		0.70139<-	
Source of Variance	**df**	**S Na^+**		**R K^+**		**S K^+**		**POD**		**SOD**		**CL**		**CFW**	
MS	P	MS	P	MS	P	MS	P	MS	P	MS	P	MS	P		
Accessions	1	5.84028 *	.0167	28.44444 **	.0080	61.36111 ***	.0002	0.000458 **	.0015	121670000000000.0 ***	.0000	78.51437 ***	.0000	3.46053 ^{ns}	.1932
Salt stress	2	643.92361 ***	.0000	562.09028 ***	.0000	619.39583 ***	.0000	0.001502 ***	.0000	2389000000000.0 ***	.0000	9.38747 ***	.0000	857.23911 ***	.0000
Mg	1	31.17361 ***	.0000	156.25000 ***	.0000	110.25000 ***	.0000	0.001236 ***	.0000	614450000000.0 ***	.0000	5.31687 **	.0048	27.45323 ***	.0009
Accession * Salt stress	2	21.79861 ***	.0000	34.46528 ***	.0006	22.29861 **	.0039	0.000105 ^{ns}	.0722	119680000000.0 ***	.0001	7.70885 ***	.0001	16.15583 **	.0018
Accession * Mg	1	0.00694 ^{ns}	.9300	0.25000 ^{ns}	.7885	0.44444 ^{ns}	.7112	0.000014 ^{ns}	.5395	5755750000.0 ^{ns}	.4017	1.12537 ^{ns}	.1659	235.84001 ***	.0000
Salt stress * Mg	2	2.09028 ^{ns}	.1150	20.02083 **	.0083	0.14583 ^{ns}	.9551	0.000001 ^{ns}	.9851	10714000000.0 ^{ns}	.2765	2.28243 *	.0285	114.56615 ***	.0000
Accession * Salt stress * Mg	2	0.04861 ^{ns}	.9465	0.14583 ^{ns}	.9580	6.13194 ^{ns}	.1661	0.000002 ^{ns}	.9470	4937940000.0 ^{ns}	.5436	1.99014 *	.0425	2.22829 ^{ns}	.3323
Error	24	0.88194<-		3.39583<-		3.16667<-		0.000036<-		7895880000.0<-		0.55101<-		1.93118<-	

Note: various abbreviations used here are as follows; SFW = shoot fresh weight, SDW = shoot dry weight, RFW = root fresh weight, RDW = root dry weight, NL = number of leaves, SL = shoot length, RL = root length, RMP = relative membrane permeability, H_2O_2 = hydrogen peroxide, MDA = malondialdehyde, TSP = total soluble proteins, LP = leaf proline,TP = total phenolics, R Na^+ = root sodium, S Na^+ = shoot sodium, R K^+ = root potassium, S K^+ = shoot potassium, POD = peroxidases, SOD = superoxide dismutase, CL = carrot length and CFW = carrot fresh weight.

respectively, as compared to untreated stressed plants. MgO-NPs significantly affected the number of leaves of carrot under salt stress (Fig. 2-A).

3.1.6. Shoot length (SL)

With respect to shoot length, remarkable difference was seen in both accessions of *D. carota*. Salt stress (40 mM and 80 mM) reduced the SL by 20% and 13%, respectively, in accession DC-03 as compared to control. However, in accession DC-90, the SL was decreased by 4% and 18% under 40 mM and 80 mM salt stress, respectively, in contrast to control. The foliar application of MgO-NPs increased the SL by 10% and 8% under salt stress of 40 mM and 80 mM, respectively, as compared to untreated stressed plants in accession DC-03. Under salt stress (40 mM and 80 mM), in accession DC-90 the SL was increased by 3% and 6%, respectively by the application of MgO-NPs as compared to untreated plants. The interaction of all three factors Accessions * Salt Stress * Mg was non-significant. Table 1 shows that the effect of salt stress and Mg was highly significant on SL (Fig. 2-B).

3.1.7. Root length (RL)

In accession DC 03, salt stress 40 and 80 mM increased the RL by 30% and 4%, respectively, as compared to control. In accession DC-90, the RL was increased by 1% under salt stress of 40 mM, in contrast to control (Fig. 2-C). In accession DC-03, MgO-NPs foliar treatment decreased RL by 17% at 40 mM salt stress but increased (1%) under 80 mM salt stress, compared to untreated stressed plants. RL was increased by 5% and 9% in accession DC-90 when treated with MgO-NPs under salt stress of 40 mM and 80 mM, respectively, as compared to untreated plants. MgO-NPs significantly affected the RL of carrot under salt stress. The interaction of all three factors Accessions * Salt Stress * Mg was significant regarding RL (Table 1).

3.2. Impression of MgO-NPs supplementation on membrane stability attributes of D. carota under salt stress

3.2.1. Relative membrane permeability (RMP)

MgO-NPs significantly affected the RMP of carrot under salt stress. The interaction of all three factors Accessions * Salt Stress * Mg was significant regarding RMP (Table 1). In accession DC-03, the mild stress (40 mM) increased the RMP by 39% and high salt stress (80 mM) by 35%, as compared to control. In accession DC-90, the relative membrane permeability was increased by 19% and 44% under salt stress of 40 mM and 80 mM, respectively, in contrast to control. Interaction of both accessions were highly significantly different from each other with respect to relative membrane permeability. In accession DC-03, MgO-NPs foliar treatment increased RMP by 13% in plants given 40 mM salt stress. Whereas MgO-NPs foliar treatment increased RMP by 5% in plants with 80 mM salt stress, as compared to untreated stressed plants. Similarly, in accession DC-09, increase of 1% and 9% was observed in plants facing 40 and 80 mM salt stress when treated with MgO-NPs, compared to stress only plants (Fig. 3-A).

3.2.2. Hydrogen peroxide (H_2O_2)

The interaction of all three factors Accessions * Salt Stress * Mg was non-significant regarding hydrogen peroxide (Table 1). In accession DC-03, the plants given 40 and 80 mM salt stress had 33% and 27% higher production of H_2O_2, respectively, compared to control plants. In accession DC-90, the hydrogen peroxide was increased by 67% and 74% under salt stress of 40 mM and 80 mM, respectively, in contrast to control. MgO-NPs had significant effect on production of hydrogen peroxide under salt stress. In accession DC-03, MgO-NPs foliar treatment reduced hydrogen peroxide by 13% in plants encountering 40 mM salt stress. Likewise, MgO-NPs foliar treatment decreased hydrogen peroxide by 25% at 80 mM salt stress, as compared to untreated stressed plants. In accession DC-90, H_2O_2 was decreased by 29% and 37% when treated with MgO-NPs under salt stress of 40 mM and 80 mM, respectively, as compared to untreated stressed plants (Fig. 3-B).

3.2.3. Malondialdehyde (MDA)

Table 1 shows that the effect of salt stress and Mg application was highly significant on MDA content of *D. carota* accessions. The interaction of all three factors Accessions * Salt Stress * Mg was non-significant. The MDA was increased by 12% under mild salt stress (40 mM), and by 18% under high salt stress (80 mM), in accession DC-03, as compared to control. However, in accession DC-90, the MDA was increased by 6% and 12% under 40 mM and 80 mM salt stress, respectively, when compared to control. The foliar application of MgO-NPs reduced the MDA by 11% and 8% under salt stress of 40 mM and 80 mM, respectively, in accession DC-03, as compared to untreated stressed plants. In accession DC-90, the MDA was decreased by 6% under 40 mM salt stress and by 8% under 80 mM salt stress with the application of MgO-NPs, as compared to untreated stressed plants (Fig. 3-C). With respect to MDA,

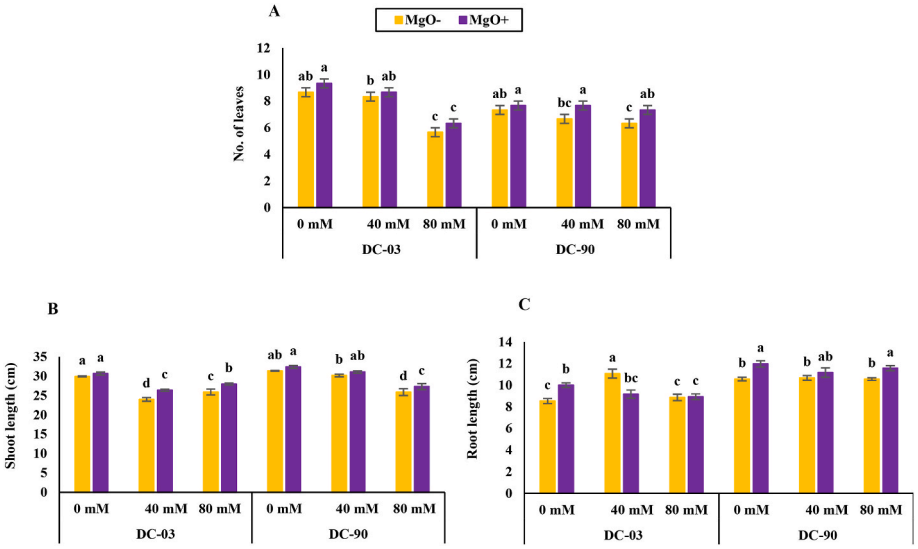

Fig. 2. Impression of MgO-NPs supplementation on number of leaves, shoot and root length and of D. carota accessions DC-03 and DC-90 under different levels of salt stress (MgO- represents untreated plants, MgO + represents plants treated with 150 mgL^{-1} MgO nanoparticles). Graph bars with same letters represent non-significant difference at $P \leq 0.05$.

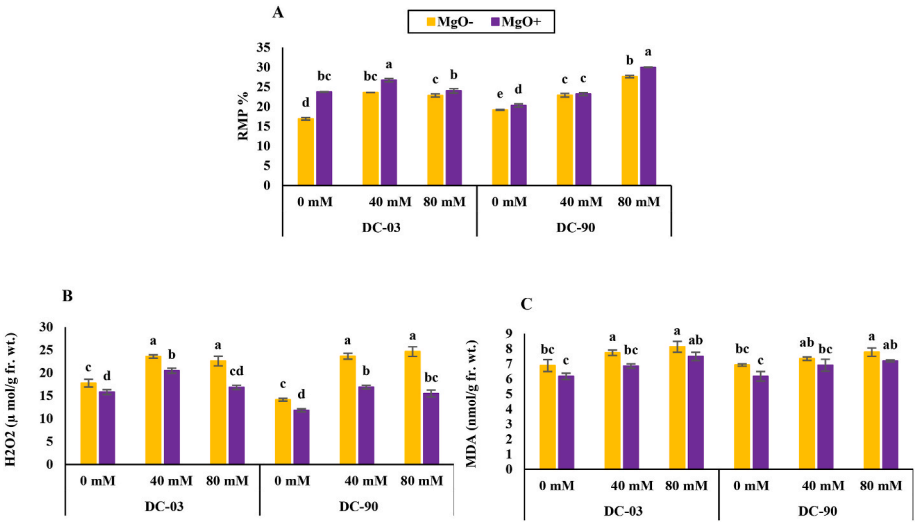

Fig. 3. Impression of MgO-NPs supplementation on Relative membrane permeability (RMP), Hydrogen peroxide (H2O2) and Malondialdehyde (MDA) of D. carota accessions DC-03 and DC-90 under different levels of salt stress (MgO- represents untreated plants, MgO + represents plants treated with 150 mgL^{-1} MgO nanoparticles). Graph bars with same letters represent non-significant difference at $P \leq 0.05$.

no remarkable difference was seen in behavior of both *D. carota* accessions (Table 1).

3.3. Impression of MgO-NPs supplementation on total soluble protein, and total phenolic of D. carota under salt stress

3.3.1. Total soluble protein (TSP)

In accession DC-03, TSP was reduced by 3% in plants encountering 40 mM salt stress. Conversely, under salt stress (80 mM) protein was increased by 12%, as compared to control. In accession DC-90, the TSP was increased by 8% under mild salt stress (40 mM). Whereas the protein was reduced (29%) at 80 mM salt stress, in contrast to control. With respect to TSP, both accessions of *D. carota* were not affected significantly in control as well as stress conditions with MgO-NPs supplementation (Table 1). The foliar application of MgO-NPs increased the protein by 16% under 40 mM salt stress, in accession DC-03, as compared to untreated stressed plants. In accession DC-90, the TSP was decreased by 4% by the application of MgO-NPs under mild salt stress (40 mM), conversely protein was increased (2%) with MgO-NPs supplementation over 80 mM salt stress, in contrast to untreated stressed plants (Fig. 4-A). Table 1 shows that the effect of salt stress and Mg was non-significant on TSP. The interaction of all three factors Accessions * Salt Stress * Mg was non-significant.

3.3.2. Total phenolic

Table 1 shows that, with respect to total phenolic, remarkable difference was seen in both accessions of *D. carota*. MgO-NPs supplementation had non-significant effect on release of total phenolic under salt stress. The interaction of all three factors Accessions * Salt Stress * Mg was highly significant (Table 1). This showed that effect of each factor on total phenolics depend upon the level of other factor. High (80 mM) stress level reduced the total Phenolic by 4% in accession DC-90, as compared to control. The foliar application of MgO-NPs decreased the total phenolic by 3% under salt stress of 40 mM, in accession DC-03, as compared to untreated stressed plants. The total phenolics reduced by 10% by the foliar application of MgO-NPs in accession DC-90, under 40 mM salt stress. Conversely, MgO-NPs foliar treatment increased total phenolic by 10% at 80 mM salt stress, compared to untreated stressed

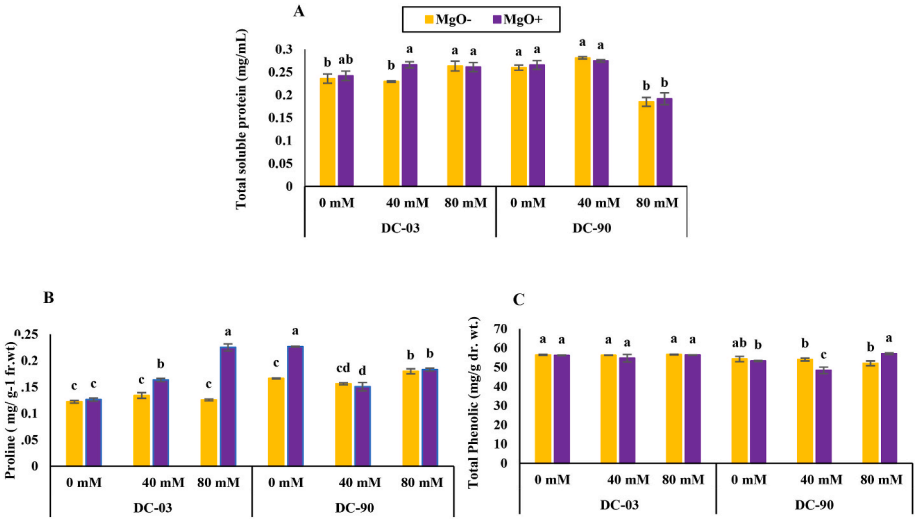

Fig. 4. Impression of MgO-NPs supplementation on Total Soluble Protein, Proline and Total Phenolics of D. carota accessions DC-03 and DC-90 under different levels of salt stress (MgO- represents untreated plants, MgO + represents plants treated with 150 mgL^{-1} MgO nanoparticles). Graph bars with same letters represent non-significant difference at $P \leq 0.05$.

plants (Fig. 4-B).

3.4. Impression of MgO-NPs supplementation on biochemical parameters of D. carota under salt stress

3.4.1. Leaf proline

. The interaction of all three factors Accessions * Salt Stress * Mg was significant regarding proline (Table 1). MgO-NPs significantly affected the proline synthesis under salt stress. Proline content was increased under both levels of salt stress when compared with control. The increase in proline content was higher (10%) in plants facing mild (40 mM) stress level, than that of plants facing high (80 mM) stress level, here only 3% increase in proline content was observed. In accession DC-90, the proline was reduced by 6% under salt stress of 40 mM, conversely at 80 mM salt stress, proline was enhanced (8%), in contrast to control. In accession DC-03, MgO-NPs foliar treatment increased proline by 22% in plants encountering 40 mM salt stress by 79% in plants given 80 mM salt stress, as compared to stressed only plants. In accession DC-90, proline was reduced by 4% when treated with MgO-NPs under salt stress of 40 mM. On the other hand, under high salt stress (80 mM) proline was increased by 2%, as compared to untreated stressed plants (Fig. 4-C).

3.5. Impression of MgO-NPs supplementation on inorganic ions of D. carota under salt stress

3.5.1. Root sodium (Na^+)

The interaction of all three factors Accessions * Salt Stress * Mg was significant (Table 1). With respect to root sodium, remarkable difference was seen in both accessions of *D. carota*. Mild (40 mM) stress level increased root Na^+ by 22% and high (80 mM) stress level by 36%, when compared control plants, in accession DC-03. However, in accession DC-90, the root sodium was increased by 82% under mild stress(40 mM) by 90% under high (80 mM) salt stress, in contrast to control. The foliar application of MgO-NPs reduced the root sodium by 11% and 4% under salt stress of 40 mM and 80 mM, respectively, in accession DC-03, as compared to untreated stressed plants. Under salt stress (40 mM and 80 m M), in accession DC-90 the root sodium was decreased by 16% and 8% by the application of MgO-NPs, as compared to untreated stressed plants. However, these plants still had high level of root Na^+ as compared to control. Table 1 shows that the effect of salt stress and Mg was highly significant on root sodium (Fig. 5-A).

3.5.2. Shoot sodium (Na^+)

In accession DC-03, shoot Na^+ was enhanced by 106% in plants encountering 40 mM salt stress by 152% in plants facing 80 mM salt stress, as compared to control. Similarly, in accession DC-90, the shoot Na^+ was increased by 100% and 104% under salt stress of 40 and 80 mM, respectively, in contrast to control. With respect to shoot Na^+, remarkable difference was seen in both accessions of *D. carota* (Table 1). In accession DC-03, the foliar application of MgO-NPs decreased the shoot Na^+ by 13% under 40 mM salt stress and by 7% under 80 mM salt stress, as compared to untreated stressed plants. In accession DC-90, the shoot Na^+ was decreased by 11% and 8% by the application of MgO-NPs, under salt stress of 40 mM and 80 mM, respectively, in contrast to untreated stressed plants (Fig. 5-B). Table 1 shows that MgO-NPs significantly affected the shoot Na^+ under salt stress. The interaction of all three factors Accessions * Salt Stress * Mg was non-significant as shown in Table 1.

3.5.3. Root potassium (K^+)

MgO-NPs significantly affected the root K^+ under salt stress. The interaction of all three factors Accessions * Salt Stress * Mg was non-significant regarding root K^+ (Table 1). The root K^+ was decreased by 19% under mild salt stress (40 mM), and by 33% under 80 mM salt stress, in accession DC-03, as compared to control. In accession DC-90, the root K^+ was decreased by 10% and 20% under salt stress of 40 mM and 80 mM, respectively, in contrast to control. In accession DC-03, MgO-NPs foliar treatment increased root K^+ by 5% and 11% in plants encountering 40 and 80 mM salt stress, when compared with stress only plants. Likewise, root K^+ was increased by 7% and 10% in accession DC-90 when treated with MgO-NPs under salt stress of 40 mM and 80 mM, respectively, as compared to untreated stressed plants (Fig. 5-C).

3.5.4. Shoot potassium (K^+)

Shoot K^+ was reduced significantly in plants facing mild (40 mM) as well as high (80 mM) stress level and the reduction was 9% and 30% respectively, as compared to control. Similarly, in accession DC-90, the shoot K^+ was reduced by 18% and 29% under salt stress of 40 mM and 80 mM, respectively, in contrast to control. The interaction of all three factors Accessions * Salt Stress * Mg was non-significant regarding shoot K^+ (Table 1). MgO-NPs significantly affected the shoot K^+ under salt stress. In accession DC-03, MgO-NPs foliar treatment increased shoot K^+ by 8% in plants encountering 40 mM salt stress and by 15% in plants with 80 mM salt stress, as compared to untreated stressed plants. Likewise, in accession DC-90, shoot K^+ was increased by 10% and 5% when

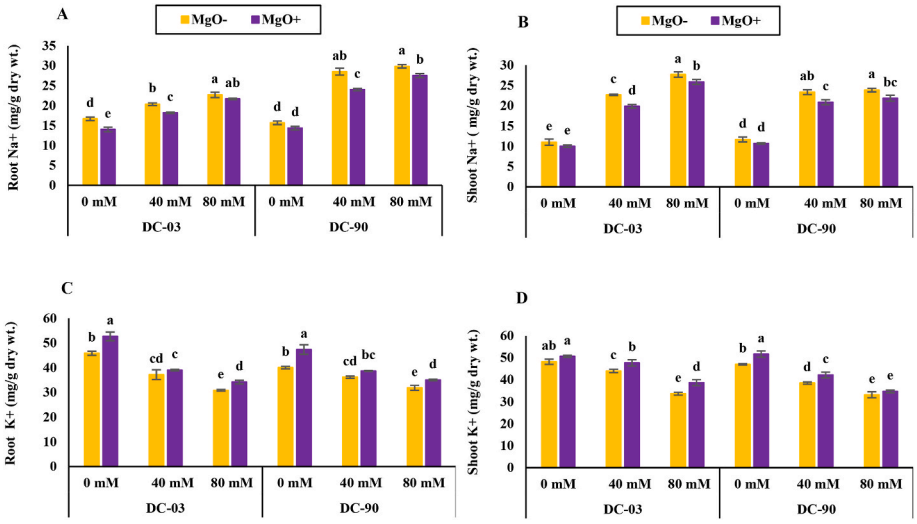

Fig. 5. Impression of MgO-NPs supplementation on Inorganic Ions of D. carota accessions DC-03 and DC-90 under different levels of salt stress (MgO- represents untreated plants, MgO + represents plants treated with 150 mgL^{-1} MgO nanoparticles). Graph bars with same letters represent non-significant difference at $P \leq 0.05$.

treated with MgO-NPs under salt stress of 40 mM and 80 mM, respectively, as compared to untreated stressed plants (Fig. 5-D).

3.6. Antioxidants

3.6.1. Activity of peroxidase (POD)

The interaction of all three factors Accessions * Salt Stress * Mg was non-significant regarding peroxidase (Table 1). But these factors had significant effect on peroxidase activity when considered individually. MgO-NPs significantly affected the peroxidase under salt stress as shown in Table 1. In accession DC-03, the mild stress (40 mM) increased the peroxidase activity by 6% and high salt stress (80 mM) by 15%, as compared to control. In accession DC-90, the peroxidase was enhanced by 15% and 25% under salt stress of 40 mM and 80 mM, respectively, in contrast to control. In accession DC-03, MgO-NPs foliar treatment enhanced peroxidase by 12% under mild salt stress (40 mM) and at 80 mM salt stress, peroxidase increased by 10%, as compared to untreated stressed plants. How-ever peroxidase was increased by 7% in accession DC-90 when treated with MgO-NPs under stress conditions, as compared to untreated stressed plants (Fig. 6-A).

3.6.2. Superoxide dismutase (SOD)

MgO-NPs significantly affected the superoxide dismutase under salt stress as shown in Table 1. The interaction of all three factors Accessions * Salt Stress * Mg was non-significant regarding superoxide dismutase (Table 1). In accession DC-03, 27% and 122% increase in SOD activity was observed in plants facing 40 and 80 mM stress level, respectively, when compared with control. Similarly, in accession DC-90, the SOD was increased by 35% and 60% under salt stress of 40 mM and 80 mM, respectively, in contrast to control. In accession DC-03, MgO-NPs foliar treatment enhanced superoxide dismutase by 41% in plants encountering 40 mM salt stress. Likewise, MgO-NPs foliar treatment increased superoxide dismutase by 20% at 80 mM salt stress, as compared to untreated stressed plants. SOD was increased by 14% and 6% in accession DC-90 when treated with MgO-NPs under salt stress of 40 mM and 80 mM, respectively, as compared to untreated stressed plants (Fig. 6-B).

3.7. Impression of MgO-NPs supplementation on yield parameter of D. carota under salt stress

3.7.1. Carrot length

The interaction of all three factors Accessions * Salt Stress * Mg was significant regarding carrot length (Table 1). The mild stress (40 mM) increased the carrot length (16%) in accession DC-03, but the length was decreased by 20% under high salt stress (80 mM), as compared to control (Fig. 6-C). In accession DC-90 (Fig. 6-C), the length was reduced by 11% and 12% under salt stress of 40 mM and 80 mM, respectively, in contrast to control. In accession DC-03, MgO-NPs foliar treatment reduced carrot length by 6% in plants encountering 40 mM salt stress. Conversely, MgO-NPs foliar treatment increased carrot length by 23% at 80 mM salt stress, as compared to untreated stressed plants. Carrot length was increased by 10% and 9% in accession DC-90 when treated with MgO-NPs under salt stress of 40 and 80 mM, respectively, as compared to untreated plants. MgO-NPs significantly affected the length of carrot under salt stress. Results of both accessions were highly significantly different from each-other with respect to carrot length as shown in Table 1.

3.7.2. Carrot fresh weight (FW)

In accession DC-03, carrot FW was reduced by 13% in plants encountering 40 mM salt stress. However, under high salt stress (80 mM) carrot FW was decreased by 41%, as compared to control. In accession DC-90, the carrot FW was decreased by 16% under mild salt stress (40 mM). The carrot FW was also reduced (53%) at 80 mM salt stress, in contrast to control. With respect to carrot FW, no remarkable difference was seen in both accessions of *D. carota* (Table 1). The foliar application of MgO-NPs decreased the carrot FW by 3% under 40 mM salt stress, in accession DC-03, conversely under 80 mM salt stress carrot FW was increased by 2%, as compared to untreated stressed plants. In accession DC-90, the carrot FW was increased by 31% and 53% by the application of MgO-NPs, under salt stress of 40 mM and 80 mM, respectively, in contrast to untreated stressed plants. Table 1 shows that MgO-NPs significantly affected the carrot FW under salt stress (Fig. 6-D). The interaction of all three factors Accessions * Salt Stress * Mg was non-significant as shown in Table 1.

3.7.3. Pearson's correlation

The Pearson's correlation analysis showed that in both varieties, stress markers (RMP, H_2O_2, MDA, POD, SOD) were in positive correlation with Na^+ concentration in root as well as shoot of plants. But these were in negative correlation with other morphological, biochemical (i.e. total phenolics, proline, total soluble proteins) and yield attributes (carrot length and fresh weight). These findings suggested that increased Na^+ level due to salinity stimulated stress markers that induces

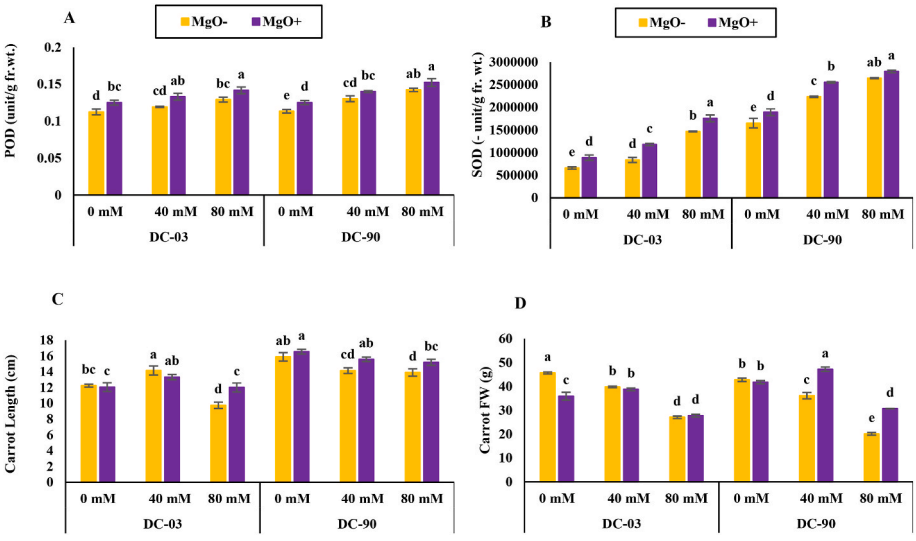

Fig. 6. Impression of MgO-NPs supplementation on Antioxidants and Yield of D. carota accessions DC-03 and DC-90 under different levels of salt stress (MgO- represents untreated plants, MgO + represents plants treated with 150 mgL^{-1} MgO nanoparticles). Graph bars with same letters represent non-significant difference at $P \leq 0.05$.

oxidative damage and ultimately hampered plant's growth and yield (Figs. 7 and 8).

4. Discussion

In our present study, salt stress impedes the growth attributes such as shoot fresh weight, shoot length, shoot dry weight, root fresh weight (only under high stress), root dry weight, and number of leaves in both carrot accessions DC-03 and DC-90 as described in previous studies in underground crops (Chourasia et al., 2022). Under salinity stress, the growth of root was inhibited by decreased cell expansion and division (M. R. Arif et al., 2019). When the salt stress imposed on plant, the first phase of stress is the phase of osmotic where the amount of soil covering the rhizosphere enhanced to threshold level and as a result shoot growth was reduced to a significant level. The major factor that affects the shoot biomass and reduces the shoot growth under salt stress is leaf abscission. This is because of accumulation of ions of chlorides in, shoot that synthesis the ethylene production aminocyclopropane-1-carboxylic acid (ACC). Thus increased production of ethylene hormone causes leaf abscission in plants (Ramón et al., 2017). In our study, the root length was increased under salt stress in accessions DC-03 of carrot as prescribed in previous studies of Arabidopsis thaliana and Silene vulgaris (Franco et al., 2008; Y. Wang et al., 2009). The root elongation is due to the cell expansion and cell division in the apical meristem of root. We can assume that salt stress may change elongation of root both by enhancing and inhibiting the cell expansion and cell division (West et al., 2004). Our results suggests that, the foliar application of MgO-NPs increased the growth parameters (shoot fresh weight, shoot length, shoot dry weight, root fresh weight, root length, root dry weight and number of leaves) in both carrot accessions DC-03 and DC-90 under salt stress, same as described in previous studies in safflower under salt stress and in soybean under arsenic stress (Faizan et al., 2022; Farhangi-Abriz and Ghassemi-Golezani, 2021). The root and shoot growth was improved by diminishing the accumulation and absorption rate of sodium in plants cell, and also by ameliorating the osmotic stress under salt stress (Farhangi-Abriz and Ghassemi-Golezani, 2021). The cell division, activation of photosynthetic enzyme ribulose 1,5 bisphosphate (RuBP) increases the number of leaves and osmoregulation by Mg which help to promote new buds and induce the new leaves (Jayarambabu et al., 2016).

It is illustrated in our study that, attributes of membrane stability including Hydrogen peroxide (H_2O_2), Relative Membrane Permeability (RMP) and Malondialdehyde (MDA) increased under abiotic stress as described earlier in *B. rapa* (Hasnain et al., 2023) carrot and radish (El-Batal et al., 2023; Taqdees et al., 2022). Lipid peroxidation produces malondialdehyde which induces the production of reactive oxygen species (ROS) such as contents of H_2O_2 which causes oxidative stress in plants (De Azevedo Neto et al., 2006; Pei et al., 2010). The ROS damage the proteins and nucleic acids and induces the lipid peroxidation, which is considered most detrimental process in all living organisms. When ROS levels reached above threshold level then lipid peroxidation occurs. (Montillet et al., 2005). The secondary oxidative stress occur due to ROS generation, which damage the membrane system by giving rise to MDA, the main mark of lipid peroxidation (Kumar et al., 2021; Lal et al., 2021a, 2021b) Our results documented that, the foliar application of MgO-NPs reduced membrane stability parameter in both carrot

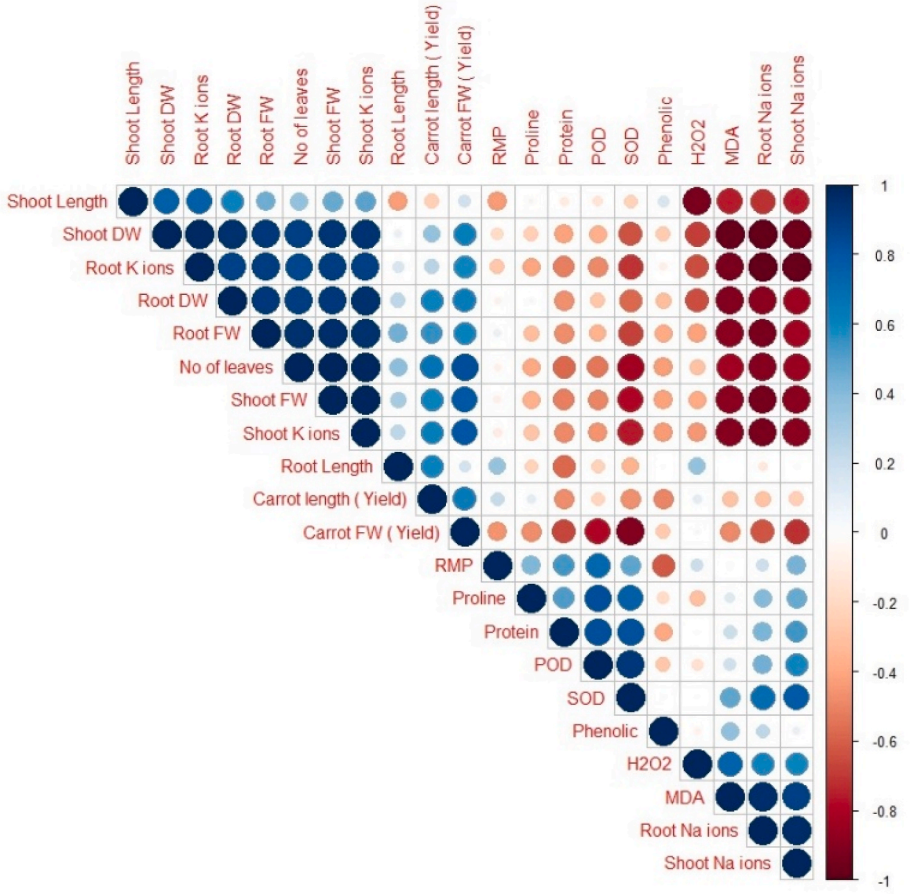

Fig. 7. Pearson correlation for all studied parameters of D. carota accession DC-03 subjected to different levels of salt stress (S1 = control, S2 = 40 mM and S3 = 80 mM) without or with MgO-NPs treatment. (Various abbreviations used are as follows, DW = dry weight, FW = fresh weight, RMP = relative membrane permeability, POD = peroxidase, SOD = superoxide dismutase, H_2O_2 = Hydrogen peroxide, MDA = Malondialdehyde).

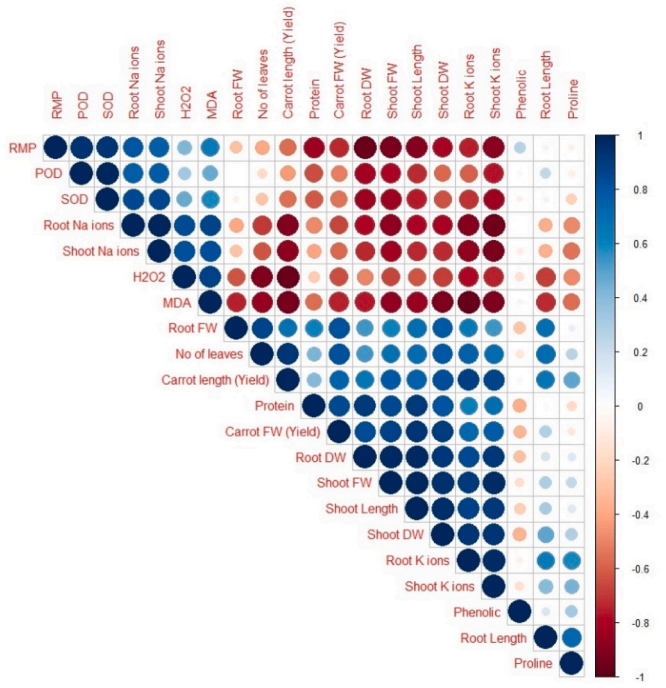

Fig. 8. Pearson correlation for all studied parameters of D. carota accession DC-90 subjected to different levels of salt stress (S1 = control, S2 = 40 mM and S3 = 80 mM) without or with MgO-NPs treatment. (Various abbreviations used are as follows, DW = dry weight, FW = fresh weight, RMP = relative membrane permeability, POD = peroxidase, SOD = superoxide dismutase, H_2O_2 = Hydrogen peroxide, MDA = Malondialdehyde).

accessions DC-03 and DC-90 as similarly prescribed in earlier researches by (Faiz et al., 2022; Kiapour et al., 2020). The basal ROS level plays a beneficial role by controlling and adjusting the ROS level in cells. There is a highly adequate antioxidant defense system in plants that overcome the oxidative stress by neutralizing, destroying and removing the free radicals. The elements of magnesium nanoparticles convert the hydrogen peroxide to water by peroxidase and catalase enzymes (Faizan et al., 2022; Kiapour et al., 2020).

It is documented by our research that, in carrot accessions (DC-03 and DC-90) salt stress enhanced the biochemical parameters consisting of leaf proline, total soluble proteins (only in DC-03), Peroxidase (POD), Superoxide dismutase (SOD) and total phenolics, as parallel to some previous reports about proline (Astaneh et al., 2018; Geng et al., 2019; Kosar et al., 2019). But the total soluble proteins in accession DC-90 reduced under salt stress as parallel to previous research in strawberry (Ghaderi et al., 2018). Plants accumulate compatible osmolytes under salt stress such as proline, for well maintenance of turgor balance used for stomatal conductance and leaf expansion. Membrane proteins, which are involved in mitigation of ion toxicity, water uptake and overall cellular homeostasis are upregulated by proline-mediated signals (Kosar et al., 2019). The enhanced production of POD and SOD helped to increase the defense response by regulating the permeability of leaf cuticle and accumulation of ROS. The plant tolerance was enhanced by higher antioxidant enzymes activities under abiotic stresses (Survila et al., 2016; Tang et al., 2006; Waris et al., 2023). The total soluble proteins are the metabolites and their amount decreased under salinity, due the accumulation of harmful ions such as Na and Cl. The content of protein might be reduced by production of increasing free radicals, protease enzymes and protein synthesis which decreases under salt stress. In plant cell free radicals increases under salinity which lead to protein decomposition (Ghaderi et al., 2018; Mäkelä et al., 1999). Our results collaborate the view that, under salt stress the foliar application of MgO-NPs increased the biochemical attributes of both carrot accession (DC-03 and DC-90) as prescribed by earlier studies (Hussain et al., 2019; Meena et al., 2019). The MgO-NPs application enhanced the activities of antioxidative enzymes by reducing the MDA content (Faizan et al., 2022). The treatment of MgO-NPs promotes the high activity of POD, which reduced the excessive H_2O_2 in cells, stabilize the structure of membrane and decreased the peroxidation effect of H_2O_2 on lipids membrane. The stimulation of activity of POD and alleviating the oxidative stress occurs due to above effects (Ding et al., 2017; Qian et al., 2016). The Mg supplementation enhanced the antioxidant activities because of Mg utilization and uptake of nutrients, which are important components of activated antioxidant systems and metabolic process in plants (Senbayram et al., 2015; Siddiqui et al., 2012). The proline serves as an osmolyte and protective function, which plays role in maintenance of redox balance through the magnesium metabolism and regulation of ROS. The metabolism of Mg improves the development and photosynthesis in plants. It is the component of different metabolic signaling networks therefore handling several stress relief, mitochondrial function and development (Meena et al., 2019).

In current study, the inorganic ions including sodium (Na^+) ions increases under salt stress in root and shoot of both carrot accessions (DC-03 and DC-90) and under salt stress potassium (K^+) ions decreases in root and shoot, in both accessions DC-03 and DC-90, as these results are parallel to some previous (Chourasia et al., 2021; Turhan and Kuscu, 2022). The increased concentration of Na^+ ions lead to prevent the uptake of K^+ ions, which is important mineral element for development and growth (Ishikawa and Shabala, 2019). Due to imbalance of concentration of ions in cell, salt stress occurs and cause the production of ROS, such as hydrogen peroxide, singlet oxygen and superoxide radicals, which interrupt the important cellular functions of plant (Heuer and Nadler, 1995; Sahoo et al., 2020; N. Zhang et al., 2011). Our study elaborated that the foliar application of MgO-NPs reduces the Na^+ ion and increased the K^+ in root and shoot of both carrot accessions DC-03 and DC-90 under salt stress as discussed earlier in safflower (Farhangi-Abriz and Ghassemi-Golezani, 2021). In rhizosphere the sodium ions have not only the highest level and interfere with the uptake of potassium in plant root, but also harm the integrity of root membrane. The highest content of potassium occurs by the application of MgO and combined use of nanocomposites which reduced the absorption rate of sodium in plants (Farhangi-Abriz and Ghassemi-Golezani, 2021).

It is documented in our research that; the salt stress impedes the yield including carrot length and fresh weight of both accessions (DC-03 and DC-90) (Chourasia et al., 2022). Under salinity stress, the growth of root was inhibited by decreased cell expansion and division (M. R. Arif et al., 2019). When the salt stress imposed on plant, the first phase of stress is the phase of osmotic where the amount of soil covering the rhizosphere enhanced to threshold level and as a result fresh weight reduced. In our study, the root length was increased under salt stress in accessions DC-03 of carrot as prescribed in previous studies of Arabidopsis thaliana and Silene vulgaris (Franco et al., 2008; Y. Wang et al., 2009). The root elongation is due to the cell expansion and cell division in the apical meristem of root. We can assume that salt stress may change elongation of root both by enhancing and inhibiting the cell expansion and cell division (West et al., 2004). Our results suggests that, the foliar application of MgO-NPs increased the yield parameters such as carrot length and fresh weight in both carrot accessions DC-03 and DC-90 under salt stress, same as described in previous studies in safflower under salt stress and in soybean under arsenic stress (Faizan et al., 2022; Farhangi-Abriz and Ghassemi-Golezani, 2021). The root growth and fresh weight was improved by diminishing the accumulation and absorption rate of sodium in plants cell, and also by ameliorating the osmotic stress under salt stress (Farhangi-Abriz and Ghassemi-Golezani, 2021).

5. Conclusion

Underground crops are sensitive and vulnerable under salt stress. These crops contain important nutritional compounds and

phytochemicals which are adversely affected due to salt stress. Salinity influences morphological and cellular interference and disproportion of nutrients which usually affects the plants. Salt stress also affect biochemical attributes and reduces the yield. In this study, we investigate the auspicious effect of magnesium nanoparticles (MgO-NPs) on the morphological and biochemical parameters, as well as the action of antioxidant enzymes in carrot (*D. carota*). In both carrot accessions (DC-03 and DC-90), foliar spray of MgO-NPs (150 mg/L) was the most effective treatment and ameliorated salt stress. The MgO-NPs improved the morphological and biochemical parameters greatly. The exposure of MgO-NPs greatly enhanced the yield. Our findings showed the ability of MgO-NPs to promote plant development and growth in salinity. However, more research is needed to investigate the efficiency of MgO-NPs as a salinity ameliorant and possible fertilizer for a variety of other plants.

Future recommendations

This research study was done in control conditions and limited work area. Further research is needed to assess real-time results in fields. MgO-NPs can be potentially used in replacement of conventional fertilizers and would prove cost effective strategy.

Funding

Researchers Supporting Project number (RSP2024R118), King Saud University.

CRediT authorship contribution statement

Hurmat Mehdi Mirrani: Investigation, Methodology, Data curation, Formal analysis. **Zahra Noreen:** Supervision, Validation. **Sheeraz Usman:** Software, Validation, Writing – original draft. **Anis Ali Shah:** Resources, Writing – original draft, Writing – review & editing. **Eman A. Mahmoud:** Funding acquisition, Writing – review & editing. **Hosam O. Elansary:** Funding acquisition, Visualization, Writing – review & editing. **Muhammad Aslam:** Supervision, Validation. **Abdul Waqas:** Software, Writing – original draft. **Talha Javed:** Software, Validation, Writing – original draft.

Declaration of competing interest

All the authors declare no conflict of interest.

Data availability

Data will be made available on request.

Acknowledgements

The completion of this research paper has been made possible through the collaborative efforts of all authors. We would like to express our heartfelt gratitude to the study participants for their invaluable contributions to this research. The authors would like to thank Researchers Supporting Project number (RSP2024R118), King Saud University, Riyadh, Saudi Arabia.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.plaphy.2024.108383.

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