Environmental Science and Pollution Research (2021) 28:42877–42890
https://doi.org/10.1007/s11356-021-13794-w

RESEARCH ARTICLE

Enhanced tolerance to salinity stress in grapevine plants through application of carbon quantum dots functionalized by proline

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Received: 22 October 2020 / Accepted: 31 March 2021 / Published online: 7 April 2021

Abstract

Salinity has destructive impacts in plant production; therefore, application of new approaches such as nanotechnology and plant priming is attracting increasing attention as an innovative means to ameliorate salt stress effects. Considering the unique properties and recorded beneficial influence of carbon quantum dots (CQDs) and proline in plant growth and physiological parameters when applied individually, their conjugation in the form of carbon quantum dot nanoparticles functionalized by proline (Pro-CQDs NPs) could lead to synergistic effects. Accordingly, an experiment was conducted to evaluate the impact of this advanced nanomaterial (Pro-CQDs NPs) as a chemical priming agent, in grapevine plants cv. 'Rasha'. For this purpose, proline, CQDs, and Pro-CQDs NPs at three concentrations (0, 50, and 100 mg L^{-1}) were applied exogenously 48 h prior to salinity stress (0 and 100 mM NaCl) that was imposed for a month. Three days after imposing salt stress, an array of biochemical measurements was recorded, while agronomic and some physiological parameters were noted at the end of the stress period. Results revealed that proline treatment at both concentrations, as well as CQDs and Pro-CQDs NPs at low concentration, positively affected grapevine plants under both non-stress and stress conditions. Specifically, the application of proline at 100 mg L^{-1} and Pro-CQDs NPs at 50 mg L^{-1} resulted in optimal performance identifying 50 mg L^{-1} Pro-CQDs NPs as the optimal treatment. Proline treatment at 100 mg L^{-1} increased leaf fresh weight (FW) and dry weight (DW); chl *a*, *b*, and proline content; SOD activity under both non-stress and stress conditions; Y (II) under salinity and carotenoid content; and CAT activity under control conditions. Pro-CQDs NP treatment at 50 mg L^{-1} enhanced total phenol, anthocyanin, and $^{Fv}/_{Fo}$, as well as APX and GP activities under both conditions, while increasing carotenoid, Y (II), $^{Fv}/_{Fo}$, and CAT activity under salinity. Furthermore, it decreased MDA and H_2O_2 contents at both conditions and EL and Y (NO) under salt stress. Overall, conjugation of CQDs with proline at 50 mg L^{-1} resulted in further improving the protective effect of proline application at 100 mg L^{-1}. Therefore, functionalization of NPs with chemical priming agents appears to be an effective means of optimizing plant-priming approaches towards efficient amelioration of abiotic stress–related damage in plants.

Responsible Editor: Gangrong Shi

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Keywords Abiotic stress · Nanoparticles · Grapevine · Chemical priming · Antioxidants

Introduction

Salt stress results in various biochemical and metabolic changes in plants leading to oxidative stress that greatly affects plant metabolism, growth, performance, and productivity. In fact, salinity causes disorders in morphological, physiological, nutritional, and biochemical characteristics of plants, ion toxicity (Na^+ and Cl^-), and osmotic stress revealed as significant decrease in plant yield (Wani et al. 2020). Plant reactions to salinity are highly variable including osmotic regulation, CO_2

assimilation, toxic ion uptake, ion compartmentation and/or exclusion, chlorophyll content, chlorophyll fluorescence, reactive oxygen species (ROS) generation, antioxidant defenses, and photosynthetic electron transport (Yan et al. 2013; Tang et al. 2015). Several studies have recently focused on promising strategies such as chemical and biological priming to deal with salinity in order to minimize its negative effects (e.g., Pollastri et al. 2018; Gohari et al. 2020a).

Advanced nanotechnology offers attractive benefits such as enhanced effectiveness of chemicals, achieved through improved nanostructure, which reduces their environmental load in chemical priming applications (Khan et al. 2017; Ioannou et al. 2020). Despite relevant lack of knowledge regarding interaction between plants and NPs, their application could be considered a promising strategy for the agricultural sector (Ioannou et al. 2020). In addition, to promote plant growth and development, NPs play an important role in plant tolerance against abiotic stresses by activating the antioxidant defense apparatus and ROS detoxification. Additionally, NPs protect the photosynthetic process, a cellular process sensitive to abiotic stresses, by diminishing osmotic and oxidative stress (Khan et al. 2017; Khan et al. 2020). Increasing attention is being given to NPs like quantum dots (QDs) due to their size-dependent fundamental properties in the range of 2–10 nm, giving them the role of a carrier (Bai et al. 2019). They additionally have distinctive fluorescent characteristics widely used in biological studies for subcellular labeling and imaging (Biju et al. 2010). QDs are considered zero-dimensional and semiconductor NPs with quantization of energy providing unique features (Bai et al. 2019). Carbon QDs (CQDs), representing a new generation of QDs in which carbonic compounds contain oxygen, demonstrate various properties commonly found in carbon-based NPs (Lim et al. 2015). They are round-shaped NPs with less than 10 nm in size providing distinctive physicochemical characteristics such as low or no toxicity and high water solubility, biocompatibility, and biodegradability with low cost and broad range of usages (Zhang et al. 2015). Several reports have demonstrated beneficial effects of different QDs in plants. For instance, treated garlic and coriander seeds with graphene QDs indicated an increase in growth parameters (e.g., leaves, roots, shoots, flowers, and fruits) (Chakravarty et al. 2015). CQDs enhanced rice yield due to increased RuBisCO enzyme activity. In addition, they improved seed germination, root elongation, carbohydrate production, and resistance to diseases through enhanced thionin gene expression (Li et al. 2018).

Proline, a strong non-enzymatic antioxidant in plant defense system inhibiting programmed cell death and ROS effects, is upregulated in plants facing abiotic stresses like salinity (Khanna-Chopra et al. 2019). Proline is an osmotic protector causing protein fixation, metal chelation, inhibition of lipid oxidation, and detoxification of ROS (Gill and Tuteja 2010). Plants produce proline and accumulate it in the cytosol, as a reaction to stresses like salinity, which helps modifying cytoplasmic osmotic properties and thus enhancing plant tolerance (Khanna-Chopra et al. 2019). Enhanced proline under salinity stress conditions acts as a deposit for extra nitrogen (N) and energy achieved by a decrease in growth following salinity that is necessary for plant survival and growth under stressful conditions (Kubala et al. 2015). Therefore, exogenous proline treatment could be beneficial in this regard and has been widely used as a priming agent towards protection of plants against osmotic stresses (e.g., Li et al. 2014). Its protective role may be through the reduction in cytotoxic Na^+ ion effect that leads to improvement in plant metabolism, decrease in lipid peroxidation of membranes, and enhancement in antioxidative enzymes (e.g., CAT, POD) under salinity stress, ultimately leading to increased cell and plant growth (Hoque et al. 2007).

Taking into account the well-demonstrated positive impacts of CQDs and proline, individually, on plant growth parameters and physiological performance, their conjugation as functionalized Pro-CQDs NPs in a nanostructure could lead to further improved properties at lower amounts. Consequently, innovative Pro-CQDs NPs were synthesized and characterized (e.g., FT-IR and TEM analysis) and subsequently applied as priming agents in salt-sensitive grapevine cv. 'Rasha', in an attempt to mitigate the damaging effects of salinity stress conditions. Consequently, Pro-CQDs NPs were synthesized using CQDs as carrier for proline to enhance its efficiency as a priming agent against salinity conditions. To our knowledge, this is the first report to develop and describe such an innovative approach in an agricultural context.

Materials and methods

Experimental site, plant materials, applied treatments, and sampling time

The experiment was conducted in the research greenhouse of the Faculty of Agriculture, University of Maragheh, Maragheh, Iran (longitude 46° 16′ E, latitude 37° 23′ N, altitude 1485 m) as factorial experiment using a completely randomized design (CRD) in three replications. Two-year-old cuttings of grapevine cv. 'Rasha' were planted in 7-kg pots containing a mixture of coco peat and medium-grain perlite in a ratio of 3:1 (each pot contained a cutting). Then, they were irrigated with half-strength Hoagland solution until at least eight leaves emerged. At that point, plants were treated with the treatments four times at 12-h intervals. The treatments (chemical priming agents) included proline, CQDs, and functionalized CQDs by proline (Pro-CQDs NPs), each at three concentrations (0, 50, and 100 mg L^{-1}) and three replications. The treatments were done in combination with Hoagland

solution into the culture media of pots. The application of last treatments was performed 48 h prior to imposition of salt stress. Precisely, 48 h later, salinity stress at two concentrations (0 and 100 mM NaCl) was imposed daily via Hoagland solution and continued for a month. Throughout salinity stress application, the culture media of the pots were washed with tap water with 5-day intervals to minimize EC and pH changes. All biochemical measurements were implemented 3 days after imposition of salt stress using fully expanded leaves. The obtained leaves were instantaneously kept into liquid nitrogen for 2 min and afterwards preserved at −80 °C freezer until cellular/biochemical measurements were carried out. Other parameters including photosynthetic parameters and pigments were investigated a month after salinity application. Pigments were examined via the same abovementioned sampling protocol, while leaf fresh and dry weights and photosynthetic parameters were assayed using fresh leaves. Three technical replications were used for each measurement. Control plants were achieved using the Hoagland solution in the same manner and treated with 0 mM NaCl and 0 mg L^{-1} of the treatments (Pro, CQDs, and Pro-CQDs NPs).

Synthesis of proline-coated carbon quantum dots

Citric acid and proline were purchased from Sigma-Aldrich (Germany). Distilled water was used in all experiments. The Fourier transform infrared (FT-IR) spectra were obtained by using a Win-Bomem spectrometer, version 3.04, Galactic Industries Corporation, over the range of 500–3500 cm^{-1}. TEM image was recorded over a transmission electron microscope (Philips CM120). UV-Vis absorption spectra were recorded on a Shimadzu UV-1800 model spectrophotometer. Photoluminescence (PL) spectrum was obtained using a Perkin Elmer L550B luminescence spectrometer.

Citric acid (2 g) and proline (0.5 g) were added in a 25-mL glass beaker containing 10 mL distilled water and stirred to form a transparent solution. The obtained solution was then transferred to a Teflon-lined autoclave chamber and heated at 200 °C for 12 h. After that, the reactor temperature was let to adjust to room temperature, and the pH value of solution was adjusted to 7 with NaOH before use. Bare carbon quantum dots were also prepared following the same procedure in the absence of proline.

Leaf fresh and dry weights

Five leaf samples were individually weighed for fresh weight (FW) and then kept in the oven (70 °C, 72 h) for dry weight (DW) measurements at the harvest stage.

Quantification of photosynthetic pigments

Leaf samples were used to measure chlorophyll (Chl) *a* and *b* and carotenoids; leaves (0.2 g) were extracted in 0.5 mL acetone (3% v/v) and then centrifuged (10,000 rpm, 10 min). The absorption of the obtained supernatant was recorded at 645 nm (Chl *b*), 663 nm (Chl *a*), and 470 nm (carotenoids) by UV-Vis spectrophotometry (UV-1800 Shimadzu, Japan) and calculated from the following equations (Sharma et al. 2012a):

$$\text{Chlorophyll } a = (19/3{*}A663 - 0/86{*}A645)\ V/100W$$
$$\text{Chlorophyll } b = (19/3{*}A645 - 3/6{*}A663)\ V/100W$$
$$\text{Carotenoids} = 100\ (A470) - 3/27(\text{mg chl } b)/227$$

Note that *V* is the solution volume of the filtrate; *A* is the light absorption in wavelength at 663, 645, and 470 nm; and *W* sample fresh weight (g).

Chlorophyll fluorescence assay

A dual-pam-100 chlorophyll fluorometer (Heinz Walz, Effeltrich, Germany) was used to measure chlorophyll fluorescence parameters including $^{Fv}/_{Fo}$, Y (NO), and Y (II). Measurements were carried out after the plants were dark-adapted for 20 min (Maxwell and Johnson 2000).

Electrolyte leakage assay

EL was measured using 0.5-cm diameter discs of fully expanded fresh leaves cut and washed three times by deionized water and then incubated in ambient temperate for 24 h as the initial electrical conductivity (EC1) using a conductivity meter (Hanna, HI98192). The samples were then and there incubated in a water bath (95 °C, 20 min) to release all electrolytes and cooled down to 25 °C, and their final electrical conductivity (EC2) was measured. The electrolyte leakage (EL) was calculated from the following equation (Lutts et al. 1995):

$$\text{EL}\ (\%) = (\text{EC1}/\text{EC2}) \times 100$$

Malondialdehyde and hydrogen peroxide content

MDA content was quantified based on the method of Stewart and Bewley (1980) with some modifications. Leaves (0.1 g) were homogenized with 2.5 mL acetic acid (10% w/v) solution. After centrifuging (15,000 rpm, 20 min), the same volume of the extract and thiobarbituric acid (0.5% w/v) in trichloroacetic acid (TCA) (20%) was transferred to test tubes for 30 min at 96 °C. Extracts were then incubated at 0 °C for 5 min, centrifuged (10,000 rpm, 5 min) and their absorbance recorded at 532 and 600 nm with the spectrophotometer (UV-1800 Shimadzu, Japan). MDA content

was calculated using the extinction coefficient of 155 $mM^{-1}cm^{-1}$ using the following equations:

$$\text{MDA}\left(\text{nmol g}^{-1}\text{ FW}\right) = [(\text{A532}-\text{A600}) \times V \times 1000/\varepsilon] \times W$$

Note that ε is the specific extinction coefficient, V is the volume of crushing medium, W is the the leaf FW, A 600 is the absorbance at 600 nm, and A 532 is the absorbance at 532 nm.

To determine H_2O_2 content, 0.2 g fresh leaves were mixed with 5 mL trichloroacetic acid (0.1% w/v) in an ice bath. After centrifuging (12,000 rpm, 15 min) and obtaining the supernatant, 0.5 mL supernatant was added to 0.5 mL potassium phosphate buffer (pH 6.8, 10 mM) and 1 mL potassium iodide (1 M), and the mixture absorbance was recorded at 390 nm. H_2O_2 content was calculated by using a standard calibration curve previously made from various H_2O_2 concentrations and expressed as μmol g^{-1} FW (Sinha et al. 2005).

Proline quantification

To assay proline content of leaf samples, a ninhydrin method was used. In this regard, 0.5 g of leaf samples was homogenized in 10 mL of 3% aqueous sulfosalicylic acid and placed in an ice bath. Then, the mixture was centrifuged (1000 rpm, 4 °C), and to 2 mL obtained supernatant, 2 mL ninhydrin acid, and 2 mL glacial acetic acid (a 1:1:1 solution) were added and finely mixed and incubated at 100 °C for 1 h. The reaction was stopped in an ice bath, and finally, 4 mL toluene was added and mixed vigorously (20 s). The mixture absorbance was recorded at 520 nm using a spectrophotometer. Different concentrations of L-proline were used for standard curve and final calculation of proline values (Bates et al. 1973).

Quantification of total phenolic and anthocyanin compounds

Total phenolic content was measured according to Xu et al. (2010) using the Folin-Ciocalteu reagent. Briefly, after digesting 0.1 g leaf sample with 5 mL 95% ethanol, the mixture was kept in dark for 24 h, and then 1 mL 95% ethanol and 3 mL distilled water were added to 1 mL of supernatant. Subsequently, 0.5 mL 50% Folin-Ciocalteu solution and 1 mL 5% sodium bicarbonate were added, and after 1 h in the dark, absorbance was recorded at 725 nm using a spectrophotometer (UV-1800 Shimadzu, Japan). The absorbance values at 725 nm were converted to total phenols and expressed as milligram gallic acid (GAE) g^{-1} FW. Different concentrations of gallic acid were used as standards.

Total anthocyanin analysis was performed according to the pH differential method. Dilutions of each sample were prepared, one with potassium chloride buffer (pH 1.0), and the other with sodium acetate buffer (pH 4.5), and the dilutions were allowed to stand for 15 min, and then the absorbance was recorded at 50 and 700 nm. The concentration of total anthocyanin was calculated according by Giusti and Wrolstad (2001) method.

Antioxidant enzyme activity assays

Total soluble proteins and antioxidant enzymes activities were assayed using leaves previously stored at −80 °C freezer. All steps of enzyme extraction were carried out at 4 °C as follows: leaves (0.5 g) were homogenized with potassium phosphate buffer (pH 6.8, 100 mM) containing 1% polyvinylpyrrolidone (PVP) and EDTA (4 mM) using magnetic stirrer for 10 min. At a later centrifuging (6000 rpm, 20 min), the supernatant was collected to evaluate catalase (CAT) Aebi (1984), ascorbate peroxidase (APX) (Nakano and Asada 1981), superoxide dismutase (SOD) (Flohe and Günzler 1984), and guaiacol peroxidase (GP) (Tang and Newton 2005) enzymatic activities. In regard to APX activity, the reaction mixture consisted of 250 μL potassium phosphate buffer, 250 μL ascorbate (1 mM), 250 μL EDTA (0.4 mM), 190 μL distilled water, 250 μL H_2O_2 (10 mM), and 0.5 mL enzyme extract. The changes in absorbance of samples at 290 nm, demonstrating enzymatic activity, were recorded and the extinction coefficient was considered 2.8 cm^{-1} $mmol^{-1}$. The reaction mixture for SOD activity consisted of 2.5 mL potassium phosphate buffer, 0.2 mL methionine (0.2 M), 0.1 mL EDTA (3 mM), nitro blue tetrazolium (NBT), 1 mL distilled water, 0.1 mL $NaCa_3$ (1.5 M), 0.1 mL riboflavin and 50 μL enzyme extract illuminating in glass tubes. The unilluminated mixtures were used as blanks. Test tubes were exposed to light by immersing in a beaker 2/3 filled with clean water, maintained at 27 °C. The increase in absorbance due to formazan formation was recorded at 560 nm. One unit of SOD was defined as the amount of enzyme that inhibited the rate of nitro blue tetrazolium reduction by 50%. The assay mixture for the estimation of GP activity comprised 1 mL potassium phosphate buffer, 250 μL EDTA, 1 mL guaiacol (5 mM), 1 mL H_2O_2 (15 mM), and 50 μL enzyme extract. The rate of change in absorbance was determined at 470 nm.

Statistical analysis

All obtained data analysis was performed by using the SAS software, and the means of each treatment were analyzed by Duncan's multiple range test at the 95% level of probability (SAS Institute Inc., ver. 9.1, Cary, NC, USA).

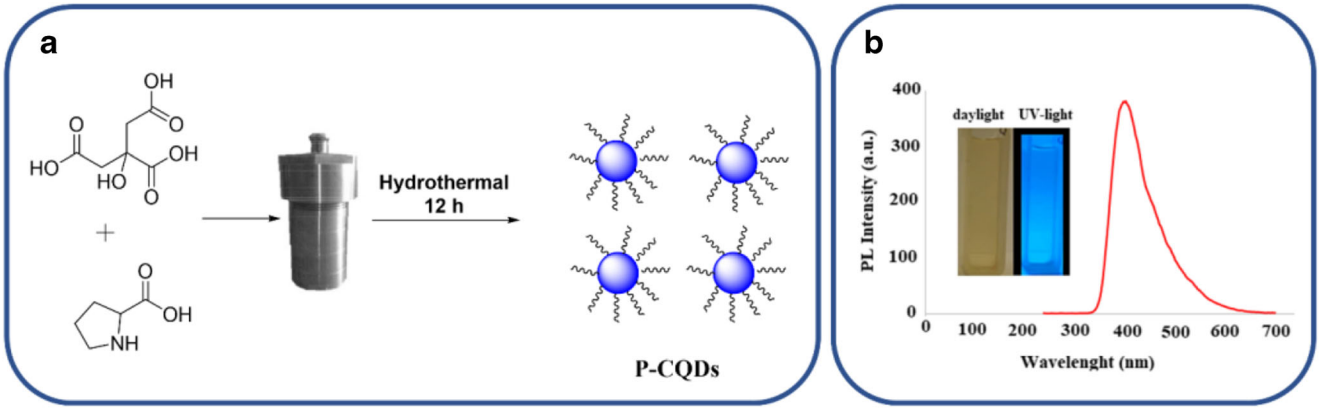

Fig. 1 Synthesis of proline-coated carbon quantum dots (Pro-CQDs NPs) using hydrothermal method (**a**), and emission spectrum of Pro-CQDs NPs in aqueous solution; inset: camera photographs of the fluorescent Pro-CQDs NPs in aqueous solution under daylight and UV-light (**b**)

Results and discussion

Synthesis and characterization of Pro-CQDs NPs

As it could be seen from Fig. 1a, a facile and one-pot hydrothermal procedure was employed for the preparation of water-stable and fluorescent proline-coated carbon quantum dots (Pro-CQDs NPs). In this method, citric acid and proline were mixed together and used as carbon source and surface coating agent, respectively. By heating the mixture at 200 °C for 12 h, citric acid was carbonized to form carbon quantum dots leading to strong blue fluorescence via surface passivation with proline. The aqueous solution of Pro-CQDs NPs illustrated blue fluorescence with λ_{max} at 400 nm under a 360-nm UV-light. Moreover, the as-prepared Pro-CQDs NPs have transparent yellow color in daylight (Fig. 1b). It should be highlighted that there was no visible precipitation of Pro-CQDs NPs even after several months. This fact could be related to the surface coating with proline to prevent CQDs from aggregation.

FT-IR spectroscopy was used to examine the composition and structural features of Pro-CQDs NPs. Characteristic bands at 3400 cm^{-1} and 2973 cm^{-1} corresponded to stretching vibration of O-H and C-H, respectively (Fig. 2a). Stretching vibration of C=O and C-N groups occurred at 1700 cm^{-1} and 1024 cm^{-1}, respectively, confirming that as-prepared Pro-CQDs NPs contain -OH and -COOH groups. Moreover, the bending vibration of N-H and stretching vibration of C-NH-C could be seen at 1557 cm^{-1} and 1400 cm^{-1}, respectively. These analyses confirmed that as-prepared Pro-CQDs NPs contained –OH and -COOH and were coated with proline.

Particle size distributions and the morphological property of Pro-CQDs NPs were further evaluated by using transmission electron microscopy (TEM) images, revealing nearly spherical particles of Pro-CQDs NPs of 5–7 nm in size (Fig. 2b).

Agronomic performance

Leaf fresh and dry weights were negatively affected by salinity. Under non-stress conditions, 100 mg L^{-1} proline and 100 mg L^{-1} CQD treatments significantly increased and decreased fresh weight, respectively. All treatments (except CQDs at 100 mg L^{-1}) increased fresh weight under salinity conditions. Dry weight was only enhanced by 100 mg L^{-1} proline under non-stress conditions. Compared with unprimed, salt-stressed grapevines, CQD treatment at 100 mg L^{-1} significantly reduced leaf dry weight indicating signs of toxicity; Pro-CQDs NP treatment at 100 mg L^{-1} concentration had no effect, while the other treatments significantly improved dry weight (Table 1).

Decrease in growth due to ionic toxicity and poor water relations could be considered the first symptom of salinity, as a mechanism to preserve carbohydrates for metabolic processes, extended energy supply and for improved recovery after stress relief (Yi et al. 2018). Osmotic stress due to high salt concentration could negatively affect plant growth and essential physiological, metabolic, and biological processes (Wani et al. 2020), thus justifying decreases in grapevine leaf FW and DW under salinity conditions. Contrarily, exogenous proline application has long been known to improve agronomic traits in plants under salt stress largely due to its osmoprotective function (e.g., Kaya et al. 2007). Interestingly, we are not aware of any studies reporting the effect of functionalized carbon quantum dots in plants under control or stress conditions. However, positive effects of other NPs on agronomic traits of plants under salinity stress were previously reported in accordance with current results (Ioannou et al. 2020; Khan et al. 2020). Furthermore, a recent report by Li et al. (2018) whereby CQDs were applied in rice plants under salt stress showed increase in FW and DW, likely due to increased tolerance resulting from improved nutrient uptake and antioxidant defense capacity. Interestingly, while application of MWCNTs-COOH improved certain agronomic traits, no effect was

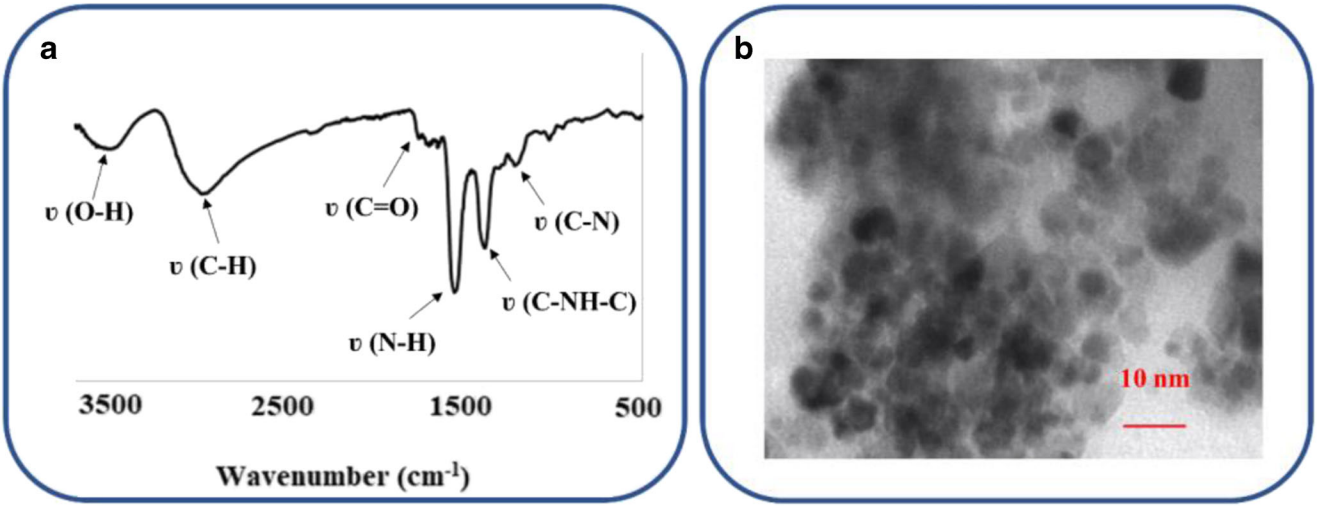

Fig. 2 FT-IR spectrum of as-prepared Pro-CQDs NPs (**a**) and TEM image of Pro-CQDs NPs (**b**)

observed in case of fresh and dry weights of basil plants (Gohari et al. 2020c).

Photosynthetic pigments

Salinity had negative effect on chl *a*, *b*, and carotenoid content. As shown in Table 1, chl *a* content was only improved by 100 mg L^{-1} proline treatment under non-stress condition. Under a salinity condition, proline treatments increased its value with the highest content at 100 mg L^{-1} concentration. Considering chl *b*, proline treatments (50 and 100 mg L^{-1}), CQDs, and Pro-CQDs NPs at 50 mg L^{-1} concentrations improved its content under non-stress condition. Under salinity conditions, a similar trend was observed with the exception of the ineffectiveness of CQDs at 50 mg L^{-1} concentration (Table 1).

Under control conditions, chl *a* content is higher than chl *b*, while salinity disturbs this balance by lowering the chl *a*/chl *b* ratio, possibly due to conversion of chl *a* to chl *b* (Mane et al. 2010). Salt stress may increase Na^+ and Cl^- concentrations in the cytosol that could in turn negatively affect enzymes and lipids in the cells due to the toxic threshold. Salt-induced inhibited CO_2 fixation might lead to leaf stomatal closure and photoinhibition, resulting in oxidative stress in the

Table 1 Effect of Pro (50 and 100 mg L^{-1}), CQDs (50 and 100 mg L^{-1}), and Pro-CQD NP (50 and 100 mg L^{-1}) treatments on key morphological parameters, photosynthetic pigments, and chlorophyll florescence parameters of *Vitis vinifera* cv. Rasha under control (0 mM NaCl) and salt stress (100 mM NaCl) conditions

NaCl (mM)	Treatments	Leaf FW (g)	Leaf DW (g)	Chlorophyll *a* (mg g^{-1} FW)	Chlorophyll *b* (mg g^{-1} FW)	Carotenoids (mg g^{-1} FW)	F_v/F_o	*Y* (II)	*Y* (NO)
0	No treatment	78.25^{bc}	7.42^{b}	1.83^{c}	0.61^{d}	92.22^{cd}	2.35^{b}	0.667^{ab}	0.386^{b}
	Pro 50 mg L^{-1}	80.02^{b}	7.08^{c}	1.87^{c}	0.82^{b}	104.65^{c}	2.16^{c}	0.674^{ab}	0.327^{e}
	Pro 100 mg L^{-1}	84.45^{a}	8.12^{a}	2.11^{a}	0.87^{a}	133.75^{a}	2.35^{b}	0.686^{a}	0.311^{ef}
	CQDs 50 mg L^{-1}	77.85^{c}	6.75^{cd}	1.54^{e}	0.76^{bc}	117.52^{b}	2.05^{cd}	0.674^{ab}	0.326^{e}
	CQDs 100 mg L^{-1}	70.22^{e}	5.19^{d}	1.06^{gh}	0.38^{f}	51.69^{g}	1.47^{ef}	0.648^{c}	0.345^{d}
	Pro-CQDs 50 mg L^{-1}	81.28^{b}	7.54^{b}	1.33^{f}	0.76^{bc}	99.29^{c}	2.33^{b}	0.675^{ab}	0.324^{e}
	Pro-CQDs 100 mg L^{-1}	78.65^{bc}	7.07^{bc}	1.11^{g}	0.38^{f}	55.39^{g}	1.48^{ef}	0.667^{ab}	0.371^{bc}
100	No treatment	60.47^{h}	4.17^{f}	1.46^{e}	0.51^{e}	75.49^{ef}	1.03^{h}	0.644^{c}	0.413^{a}
	Pro 50 mg L^{-1}	68.05^{f}	4.85^{e}	1.65^{d}	0.82^{b}	82.94^{e}	2.00^{cd}	0.669^{ab}	0.335^{de}
	Pro 100 mg L^{-1}	72.08^{d}	5.51^{d}	1.98^{b}	0.89^{a}	99.74^{c}	2.57^{a}	0.683^{a}	0.306^{f}
	CQDs 50 mg L^{-1}	63.57^{g}	4.82^{e}	1.31^{f}	0.53^{e}	82.25^{e}	1.8^{d}	0.672^{ab}	0.328^{e}
	CQDs 100 mg L^{-1}	59.19^{h}	3.92^{g}	1.02^{gh}	0.40^{f}	58.65^{g}	1.43^{ef}	0.645^{c}	0.351^{cd}
	Pro-CQDs 50 mg L^{-1}	69.51^{e}	4.98^{e}	1.51^{de}	0.63^{d}	117.03^{b}	2.52^{a}	0.674^{ab}	0.296^{g}
	Pro-CQDs 100 mg L^{-1}	71.29^{de}	4.25^{f}	1.06^{gh}	0.43^{f}	84.16^{e}	1.48^{ef}	0.621^{d}	0.365^{c}

*Different letters indicate significant differences based on Duncan's post hoc analysis at $P \leq 0.05$

chloroplast (Yan et al. 2013). Accumulation of toxic ions in chloroplasts and enhanced oxidative stress in plants by salinity stress leads to breakdown of photosynthetic pigments (Hatami 2017). Decrease in photosynthetic pigments after imposing salinity could be due to salinity effects in chloroplast membranes that reduce photosynthetic rate and subsequently plant growth (Ahmad et al. 2019). In addition, a positive effect of proline treatment on pigment content was previously reported under salt stress conditions (Yan et al. 2011; Kaya et al. 2007), also in agreement with current results, with increasing proline concentration leading to higher chl *a*. CQD treatments enhanced chl *a*, *b*, and total chlorophyll. However, increase in CQD concentrations resulted in lower values indicative of chlorophyll breakdown due to toxicity (Wang et al. 2018). In regard to the effect of salinity on carotenoid content, there are controversial reports; some observed decreased amounts (e.g., Doganlar et al. 2010; Mane et al. 2010), while others observed increases in amount (Lim et al. 2012). Enhancement in carotenoids increases plant tolerance to stress condition due to quenching of ROS (Doganlar et al. 2010). Previously, a positive effect of proline treatment on carotenoid content was reported (Zouari et al. 2016) in line with present results. In a similar fashion to effects on agronomic parameters, no previous reports show effects of CQDs on carotenoid content in plants. Confident influence of some NPs on carotenoids was noticed as well (Gohari et al. 2020c).

Chlorophyll fluorescence

Salinity exerted a negative effect on chlorophyll fluorescence parameters. Most treatments decreased $^{Fv}/_{Fo}$ parameter under non-stress condition except proline at 100 mg L^{-1} and Pro-CQDs NPs at 50 mg L^{-1} with no effect as compared with control samples. However, results were different under stress condition as all treatments positively affected this parameter with the best results being achieved at 100 mg L^{-1} proline and 50 mg L^{-1} Pro-CQDs NP treatments. Y (II) parameter was not affected by any treatment under normal condition except 100 mg L^{-1} CQDs that significantly decreased it. The treatments confidently influenced Y (NO) parameters under normal condition compared with controls. Under stress condition, 100 mg L^{-1} proline and 50 mg L^{-1} Pro-CQDs NPs had positive effect in this regard (Table 1).

Salinity changes chloroplast structure and then chlorophyll content. The severity of the changes depends on salinity dose. Plant capability at balancing ion distribution through cell (i.e., vacuole, chloroplast, and cytoplasm) determines photosynthesis rate and then its tolerance to salinity stress (Mane et al. 2010). Thus, a noteworthy decrease in chlorophyll fluorescence parameters may be caused by the dissipation of a major proportion of light energy as heat (Omoto et al. 2009) and reduced efficiency of energy transfer from antennae to the reaction centers under salt stress (Ahmad et al. 2019). A decrease in chlorophyll fluorescence parameters was previously reported under salinity (Ahanger et al. 2018; Ahmad et al. 2019). Salinity decreases stomatal conductance and the mesophyll conductance to CO_2 through disturbed water relations or abscisic acid synthesis, leading to reduced photosynthetic rate and PSII activity (Yan et al. 2013). As mentioned previously, proline treatment had a positive effect in photosynthetic pigments (Yan et al. 2011) that might describe encouraging the effect of proline-contained treatments on chlorophyll fluorescence parameters. Moreover, CQD treatments enhanced chl *a*, *b*, and total chlorophyll though in a dose-dependent manner (Wang et al. 2018). Damages in organelles and chloroplast after high concentration of graphene oxide application decreased chlorophyll biosynthesis (Hu et al. 2014). Some information indicated that chlorophyll fluorescence parameter adjustment subsequent NP application decreased $^{Fv}/_{Fm}$ by Cr_2O_3 NPs (Li et al. 2018) or increased photosynthetic quantum yield by Ag NPs (Sharma et al. 2012b); however, all occurred in a dose-dependent manner.

Electrolyte leakage

As expected, salinity enhanced EL value. This increasing trend in EL value was only observed by application of 100 mg L^{-1} CQD treatments under non-stress condition; Pro-CQDs NPs at 100 mg L^{-1} acted the same as the control, while the other treatments significantly decreased the value with the maximum decrease in 100 mg L^{-1} proline treatment. Under salinity conditions, all treatments significantly decreased grapevine EL values compared with samples under salinity with no priming treatment with the sole exception of 100 mg L^{-1} CQDs which exerted no effect. The lowest EL value was obtained by Pro-CQDs NPs at 50 mg L^{-1} concentration (Fig. 3a).

Cell membrane integrity plays a key role in plant protection against stresses. Therefore, any damage could be identified via EL value as an obvious character of cell response to stress conditions (Gohari et al. 2020b, c). Increase in EL was previously reported after imposing salinity due to salinity damages to membrane integrity (e.g., Ahmad et al. 2019; Ahanger et al. 2018). Proline application reduced EL value of grapevine under oxidative stress through increase in POD and APX antioxidant enzyme activities that enhanced ROS quenching and decreased H_2O_2 and MDA content, membrane damages, and EL value (Ozden et al. 2009). Increase in EL value was noticed after application of high concentrations of graphene oxide NPs due to damages to membrane integrity and permeability and cellular structures (Hu et al. 2014). Gohari et al. (2020c) and Hatami (2017) reported dose-dependent effects of MWCNTs and SWCNTs, respectively on EL value, the lower concentration of NPs resulting in lower EL value and vice versa, in agreement with current findings. Enhanced membrane rupture and ROS overproduction might be

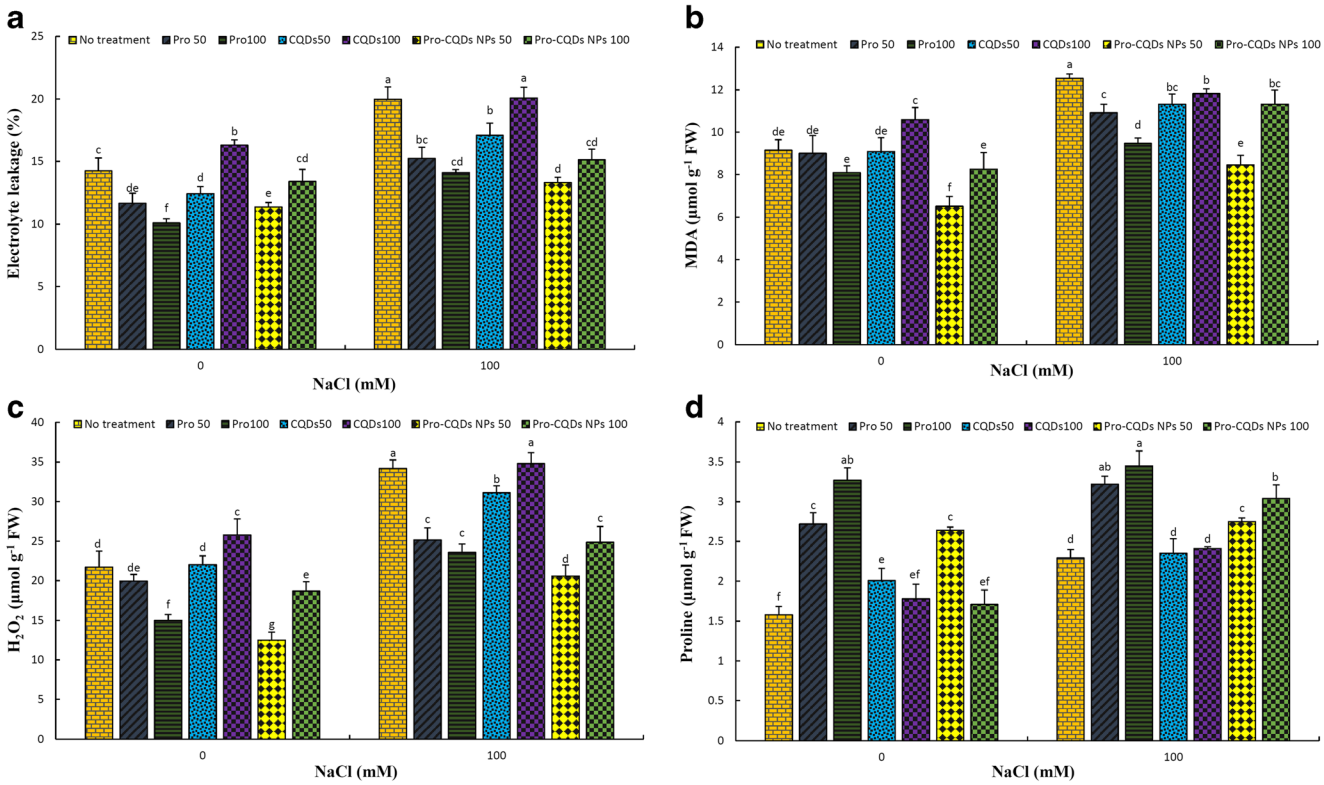

Fig. 3 Effect of Pro (50 and 100 mg L^{-1}), CQDs (50 and 100 mg L^{-1}), and Pro-CQDs NP (50 and 100 mg L^{-1}) treatments on EL (**a**), MDA (**b**), H_2O_2 (**c**), and proline (**d**) of *Vitis vinifera* cv. 'Rasha' under control (0 mM NaCl) and salt stress (100 mM NaCl) conditions. Different letters indicate significant differences based on Duncan's post hoc analysis at $P \leq 0.05$

considered probable reasons for EL increase at high concentrations of the NPs. Decrease in EL might occur via increased ROS scavenging and higher membrane stability.

MDA and H_2O_2

Salinity enhanced MDA and H_2O_2 content. Under non-stress conditions, 50 mg L^{-1} Pro-CQDs NPs significantly increased MDA content, while 100 mg L^{-1} CQDs decreased MDA content, with all other treatments not showing any effect. All treatments were effective at MDA reduction under salinity condition (Fig. 3b). The applied treatments, except 100 mg L^{-1} CQDs, mostly decreased H_2O_2 content or had no effect under non-stress condition. Under stress conditions, all treatments decreased H_2O_2 content, except 100 mg L^{-1} CQD treatment with no effect compared with grape under salinity with no priming treatment (Fig. 3c). The optimal result for MDA and H_2O_2 content was observed at 50 mg L^{-1} Pro-CQDs NPs under both conditions.

Oxidative stress under abiotic stress conditions is known to have irreversible impacts through lipid peroxidation that disturbs membrane integrity and enhances MDA content (Hasanuzzaman et al. 2020). MDA, as the last product of lipid peroxidation during salinity stress, could demonstrate oxidative stress or ROS-induced damage to membranes (Ahammed et al. 2018). Consequently, salinity is known to enhance MDA content (e.g., Ahmad et al. 2019; Khan et al. 2020). Proline application decreased ROS and MDA content of plant under salt stress (Hayat et al. 2012). A reduction in MDA content by proline application was previously reported (Quan et al. 2008; Yan et al. 2011). Furthermore, graphene QD application at low doses decreased MDA content (Feng et al. 2019). Likewise, Khan et al. (2020) reported a positive effect of AgNPs on decreasing MDA content due to recovery of membrane damage and maintaining membrane integrity, mostly in parallel with present findings.

H_2O_2 has binary effects depending on its concentration; first, at low concentrations, it acts as a signaling molecule needed for initiation of resistance mechanisms to biotic and abiotic stresses. Second, at high concentrations, it leads to oxidative stress (Molassiotis and Fotopoulos 2011), PCD, inactivation of enzymes, and lipid peroxidation through hydroxyl radical formation (Gill and Tuteja 2010). Salinity mostly resulted in increased H_2O_2 content (Ahammed et al. 2018; Ahanger et al. 2018; Khan et al. 2020), probably by excessive amount of Na^+ ions that result in nutrient imbalance and ultimately in ROS accumulation (Khan et al. 2020). Current results demonstrated a decrease in H_2O_2 value by proline

priming in accordance with previous findings (Perveen and Nazir 2018). However, Feng et al. (2019) reported a linear increase in H_2O_2 by graphene QD application. Increase in proline content by the priming treatment could result in lower H_2O_2 values as increased proline could reduce H_2O_2 and other radicals either directly or by activating antioxidant enzyme activities (e.g., SOD, APX, GP, and CAT) (Reddy et al. 2015). Accordingly, current NP treatments could improve plant performance under salt stress conditions by ameliorating oxidative stress and lessening membrane damage.

Proline

Salinity increased proline content. With the exception of CQDs and Pro-CQDs NP treatments at 100 mg L^{-1} concentrations, other treatments enhanced proline content under non-stress conditions. Under salinity stress, exogenous proline and Pro-CQDs NP treatments (50 and 100 mg L^{-1}) significantly enhanced endogenous proline content, while CQD treatments had no effect compared with unprimed, salt-stressed samples. The highest proline content was observed following 100 mg L^{-1} proline treatment (Fig. 3d). Physiological mechanisms of salt tolerance result in lessening toxic ion accumulation in plant tissues through partitioning in the apoplast and vacuole and enhancing osmoprotective metabolite biosynthesis such as proline to maintain tissue water status, additionally increasing antioxidant capacity to prevent the occurrence of oxidative stress (Yan et al. 2013). In fact, accumulation of proline as an osmolyte could avoid osmotic effects of salt, maintaining the flow of water into cells. Proline plays a key role in lowering or balancing the osmotic potential of intracellular and extracellular ions to tolerate osmotic stress that maintains water relation in plant tissue under salinity (Yi et al. 2018). Proline could act as an osmolyte, and a metal chelating, antioxidant, and signaling molecule. With low molecular weight, proline accumulates in cytoplasm under abiotic stresses, preserves cytosol pH, and modulates cellular redox resulting in compatibility responses to the stress (Hayat et al. 2012). It regulates osmotic pressure, detoxifies ROS, and preserves membrane integrity, subcellular structures, antioxidant enzyme activities, and protein structure leading to plant protection against stress (Ozden et al. 2009; Hayat et al. 2012). Proline increase under salinity could be described through the decrease in proline oxidation and the increase in its biosynthesis from glutamate or protease enzyme previously reported by Khan et al. (2020). Proline increase under salinity could maintain water status of plant under salinity (Yan et al. 2013). Application of proline enhanced endogenous proline in the plant under salinity (e.g., Perveen and Nazir 2018), in line with our findings. QD application enhanced proline content possibly through a similar reason, as well as (Feng et al. 2019) observed in current survey under non-stress condition.

Total phenolic and anthocyanin content

Current findings demonstrated that all treatments resulted in significant enhancement in total phenolics under non-stress and stress conditions, with the exception of 100 mg L^{-1} CQDs. Maximum contents were observed in 50 mg L^{-1} Pro-CQDs NP–treated grapevines under both conditions; however, the increase under salinity condition was higher than that under normal conditions (Fig. 4a). Under non-stress condition, all treatments (except 100 mg L^{-1} CQDs) significantly enhanced anthocyanin content. The highest total anthocyanin content was achieved in grapevines treated with 50 mg L^{-1} Pro-CQDs NPs. Under salinity condition, the highest content was observed at 50 mg L^{-1} Pro-CQDs NPs, as well; 50 mg L^{-1} proline caused significant decrease in anthocyanin content, and 100 mg L^{-1} CQDs had similar content as compared to grapevines under salinity without any treatment. (Fig. 4b).

A key protective mechanism for plant cells is phenolics content. Phenolics act as antioxidants by quenching ROS and free radicals. Consequently, phenolics inhibit oxidative stress and lessen its negative effects (Ashraf et al. 2010; Yan et al. 2013). Most phenolics are produced through phenylpropanoid pathway stimulated under biotic and abiotic stresses (Lim et al. 2012). This stimulation in phenolics biosynthesis was previously confirmed, especially under salt stress (Ashraf et al. 2010) and in line with current findings. Low concentrations of QDs increased phenolics, while high concentrations of NPs decreased their content (Feng et al. 2019). In addition, MWCNTs-COOH treatment enhanced plant phenolics under salinity condition probably via enhanced biosynthesis, as a line of antioxidant defense against oxidative stress imposed by NaCl (Gohari et al. 2020c). AgNP application enhanced total phenolics under salinity (Khan et al. 2020). Zouari et al. (2018) demonstrated that proline application enhanced total phenolic compounds of olive under lead stress in accordance with our findings.

Anthocyanins, as a subclass of flavonoids, play essential roles in plant tolerance to salt stress. They accumulate in vegetative tissues of plants likely leading to ROS quenching, photoprotection, stress signaling, and xenohormesis (Kovinich et al. 2015). A direct relation was noticed among enhanced anthocyanins and antioxidative enzyme activities. They prevent ROS generation and reduce oxidative stress effects via accumulation in epidermal cells (Eryilmaz 2006). Accordingly, the reported increase in anthocyanin content could contribute, at least in part, to the amelioration of salinity's toxic effects, while the decrease observed in anthocyanin content following application of high dose of CQDs could be attributed to NPs' toxicity effects that reduce anthocyanin biosynthesis and accumulation.

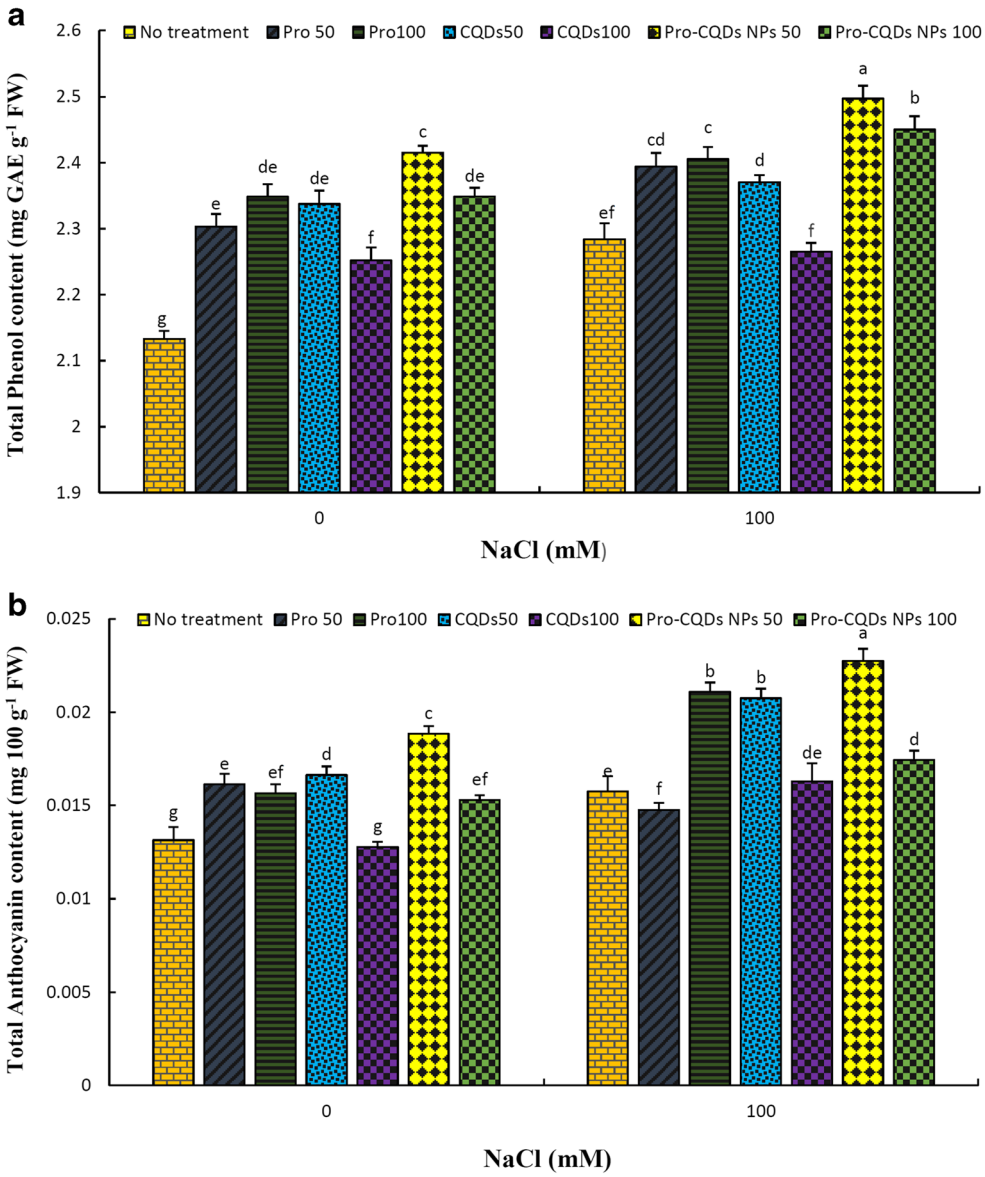

Fig. 4 Effect of Pro (50 and 100 mg L^{-1}), CQDs (50 and 100 mg L^{-1}), and Pro-CQDs NP (50 and 100 mg L^{-1}) treatments on total phenol content (**a**), and total anthocyanin content (**b**) of *Vitis vinifera* cv. 'Rasha' under control (0 mM NaCl) and salt stress (100 mM NaCl) conditions. Different letters indicate significant differences based on Duncan's post hoc analysis at $P \leq 0.05$

Antioxidant enzymes activities

Salinity increased antioxidant enzyme activities including CAT, SOD, APX, and GP. Considering CAT (Fig. 5a) and APX (Fig. 5b) enzyme activities, all treatments enhanced their activities under non-stress and stress conditions. In addition, treatments significantly enhanced the activity of SOD (Fig. 5c) and GP (Fig. 5d) enzymes under non-stress condition compared with control samples. A similar trend was observed under stress conditions with the exception of both CQD treatments for SOD and 100 mg L^{-1} CQDs for GP activity. The highest induction levels were recorded at 50 mg L^{-1} Pro-CQDs NPs under both conditions.

Antioxidant enzymes (e.g., CAT, SOD, GP, and APX) are part of a complex defense mechanism for ROS detoxification that reduces stress impacts. CAT, as the main enzyme for H_2O_2 detoxification under biotic and abiotic stress (Gharsallah et al. 2016), reduces oxidative stress damages. Since salt stress increases H_2O_2 generation through respiration and photosynthesis, CAT converts these H_2O_2 molecules to water leading to decreased toxic effects and increased plant tolerance to stress conditions (Quan et al. 2008). APX activity is similar to CAT; the enzyme removes H_2O_2 using ascorbate in the glutathione-ascorbate cycle (Gharsallah et al. 2016; Rao and Shekhawat 2016). APX efficiency in H_2O_2 detoxification is higher than that of CAT since it could react with H_2O_2 at lower concentrations, demonstrating that CAT and APX enzymes have distinct roles in ROS quenching (Rao and Shekhawat 2016). SOD converts superoxide radicals to H_2O_2 that are subsequently detoxified via peroxidase (POD) and GP enzymes. GP in turn detoxifies H_2O_2 by donating an

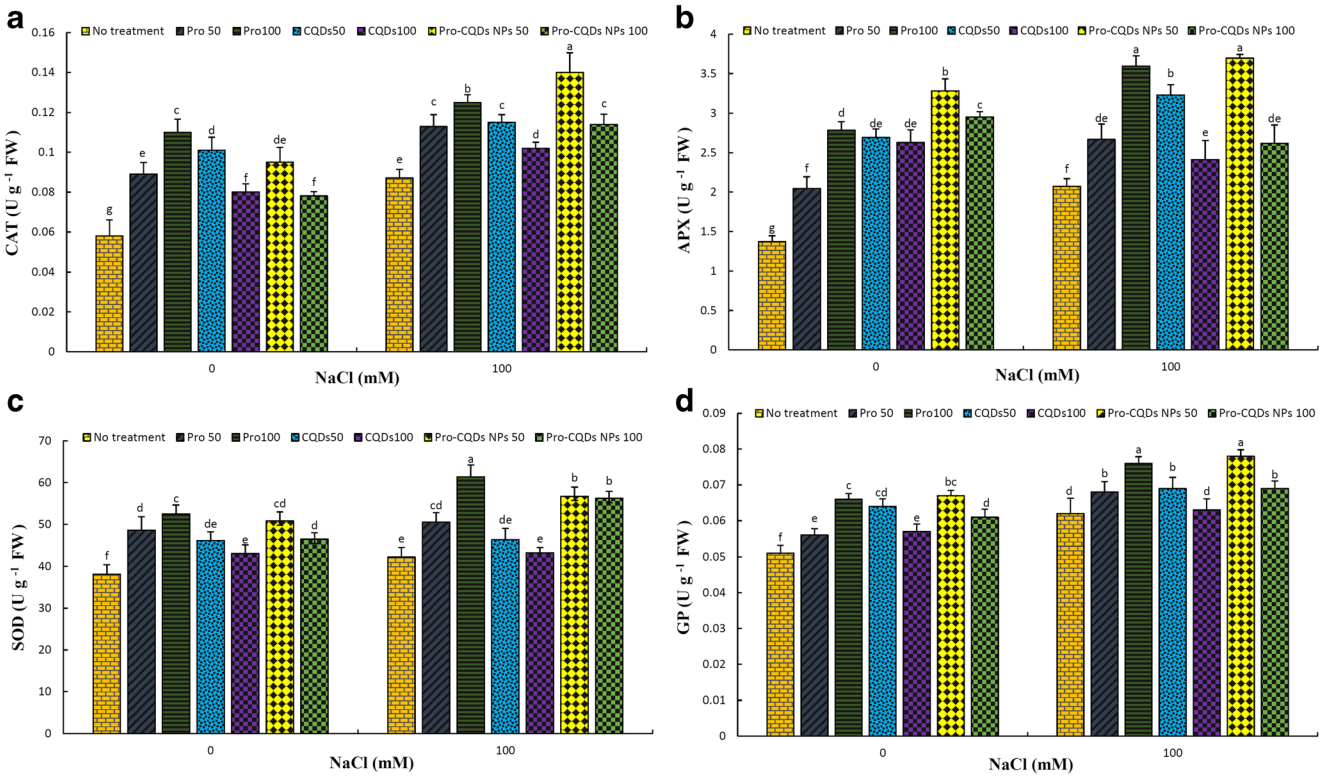

Fig. 5 Effect of Pro (50 and 100 mg L^{-1}), CQDs (50 and 100 mg L^{-1}), and Pro-CQDs NP (50 and 100 mg L^{-1}) treatment on CAT (**a**), SOD (**b**), APX (**c**), and GP (**d**) enzyme activities of *Vitis vinifera* cv. 'Rasha' under control (0 mM NaCl) and salt stress (100 mM NaCl) conditions. Different letters indicate significant differences based on Duncan's post hoc analysis at $P \leq 0.05$

electron to it and other ROS and lessens their destructive effects in cells (Gharsallah et al. 2016). An increase in antioxidant enzyme activities has been widely reported in plants under salt stress conditions (e.g., Filippou et al. 2014; Ahmad et al. 2019; Gohari et al. 2020b, c; Khan et al. 2020). Consequently, an increase in antioxidant enzyme activity could minimize the adverse effects of salt-induced oxidative stress by preventing lipid peroxidation (Ahammed et al. 2018). The increase in antioxidant enzymes\activities could quench ROS and protect the cells from oxidative damage induced by salinity stress (Ahmad et al. 2019). Proline could modulate enzyme activities related to ascorbate-glutathione cycle (Hayat et al. 2012); for instance, APX activity, as one of the enzymes involved in the cycle, increased by its application in tobacco cultures under salt stress conditions (Hoque et al. 2007). In fact, proline could initiate antioxidant enzyme activities specifically under salinity conditions to remove H_2O_2 and ROS and diminish their destructive effects. Proline treatment enhanced the activity of CAT (Yan et al. 2011), APX (Ozden et al. 2009; Zouari et al. 2016), SOD (Yan et al. 2011), and GP (Gharsallah et al. 2016; Zouari et al. 2016) enzymes under normal or salinity conditions, in line with the present findings. Gohari et al. (2020b, c) and Khan et al. (2020) reported positive effects of specific NP application on antioxidant enzyme activities under salinity conditions in agreement with the current findings. A dose-dependent effect of CQD treatments (alone or in combination with proline) on CAT activity was observed, in agreement with findings by Feng et al. (2019). Some nanocarbons like graphene oxide could modify antioxidative enzyme activities (e.g., SOD, CAT, POD) as high concentrations of nanocarbons could react with special site of enzymes and deactivate them as observed in the current study (CQDs at high concentration). The protective effects of nanomaterials are commonly attributed to their small size and high permeability to plant cells that interrupt stressful factors (Ioannou et al. 2020). In addition, NP application might decrease the uptake of Na^+ and oxidative stress and enhance the antioxidant apparatus including antioxidant enzyme activities. Since K^+ could be involved in the activation of enzymes, NPs could also enhance their content by reducing Na^+ concentration under salinity, thus explaining a positive effect of NPs on antioxidant enzymes (Khan et al. 2020). Therefore, an increase in all antioxidant enzyme activities by Pro-CQDs NP treatment could ameliorate the negative impacts of salinity.

Conclusion

Salinity decreases plant performance by reducing growth, chlorophyll pigments, and chlorophyll fluorescence parameters; altering proline, phenolics, anthocyanins, and antioxidant enzymes;

and increasing MDA, H_2O_2, and EL. Functionalized carbon quantum dot nanoparticles by proline (Pro-CQDs NPs) were successfully applied as priming agents to alleviate the salinity effects on grapevine cv. "Rasha". The positive effects of Pro-CQDs NPs were highlighted through the increase in leaf FW and DW, photosynthetic pigments, chlorophyll fluorescence parameters; proline, phenolic, and anthocyanin contents; and antioxidant enzyme activities as well as decrease in EL, MDA, and H_2O_2. CQDs, particularly at high concentrations, established toxic effect or ineffectiveness; however, CQDs demonstrated positive effects when conjugated with proline (Pro-CQDs NPs), likely due to them being nanosized and have improved proline delivery using CQDs as a carrier. In addition, even though the current study reported a positive effect of proline at higher concentrations at ameliorating salinity effects, its conjugation with CQDs resulted in further improved performance at lower doses highlighting 50 mg L^{-1} Pro-CQDs NPs as the optimal treatment to ameliorate salinity effects. Lastly, Pro-CQDs NPs could therefore be applied as innovating priming agents in grapevine cv. "Rasha" to enhance plant tolerance to salinity.

Abbreviations APX, Ascorbate peroxidase; CAT, Catalase; Chl, Chlorophyll; CQDs, Carbon quantum dots; DW, Dry weight; FW, Fresh weight; GP, Guaiacol peroxidase; H_2O_2, Hydrogen peroxide; MDA, Malondialdehyde; Pro, Proline; Pro-CQDs NPs, Carbon quantum dots nanoparticles functionalized by proline; SOD, Superoxide dismutase

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11356-021-13794-w.

Acknowledgements S. Panahirad acknowledges University of Tabriz (Tabriz, Iran) for the grant research support (grant number 4602). The authors express sincere thanks to the University of Maragheh for providing the research facilities to carry out the present research study.

Author contribution GG, SP, VF, and AA designed the experimental setup. NS and AF performed greenhouse experiments, and applied biochemical and physiological parameters. AA and HJ synthesized the nanomaterials. SMZ performed statistical analysis. GG, MD, and AA analyzed data and results, while GG, SP, and VF wrote the manuscript.

Data availability The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate Not applicable.

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

References

Aebi H (1984) Catalase in vitro. Methods Enzymol 105:121–126

Ahammed GJ, Li Y, Li X, Han WY, Chen S (2018) Epigallocatechin-3-gallate alleviates salinity-retarded seed germination and oxidative stress in tomato. J Plant Growth Regul 37(4):1349–1356

Ahanger MA, Alyemeni MN, Wijaya L, Alamri SA, Alam P, Ashraf M, Ahmad P (2018) Potential of exogenously sourced kinetin in protecting Solanum lycopersicum from NaCl-induced oxidative stress through up-regulation of the antioxidant system, ascorbate-glutathione cycle and glyoxalase system. PLoS One 13(9):e0202175

Ahmad P, Ahanger MA, Alam P, Alyemeni MN, Wijaya L, Ali S, Ashraf M (2019) Silicon (Si) supplementation alleviates NaCl toxicity in mung bean [*Vigna radiata* (L.) Wilczek] through the modifications of physio-biochemical attributes and key antioxidant enzymes. J Plant Growth Regul 38(1):70–82

Ashraf MA, Ashraf M, Ali Q (2010) Response of two genetically diverse wheat cultivars to salt stress at different growth stages: leaf lipid peroxidation and phenolic contents. Pak J Bot 42(1):559–565

Bai X, Purcell-Milton F, Gun'ko YK (2019) Optical properties, synthesis, and potential applications of Cu-based ternary or quaternary anisotropic quantum dots, polytypic nanocrystals, and core/shell heterostructures. Nanomaterials 9(1):85

Bates LS, Waldren RP, Teare ID (1973) Rapid determination of free proline for water-stress studies. Plant Soil 39(1):205–207

Biju V, Mundayoor S, Omkumar RV, Anas A, Ishikawa M (2010) Bioconjugated quantum dots for cancer research: present status, prospects and remaining issues. Biotechnol Adv 28(2):199–213

Chakravarty D, Erande MB, Late DJ (2015) Graphene quantum dots as enhanced plant growth regulators: effects on coriander and garlic plants. J Sci Food Agric 95(13):2772–2778

Doganlar ZB, Demir K, Basak H, Gul I (2010) Effects of salt stress on pigment and total soluble protein contents of three different tomato cultivars. Afr J Agric Res 5(15):2056–2065

Eryilmaz F (2006) The relationships between salt stress and anthocyanin content in higher plants. Biotechnol Biotechnol Equip 20(1):47–52

Feng P, Geng B, Cheng Z, Liao X, Pan D, Huang J (2019) Graphene quantum dots-induced physiological and biochemical responses in mung bean and tomato seedlings. Brazil J Bot 42(1):29–41

Filippou P, Bouchagier P, Skotti E, Fotopoulos V (2014) Proline and reactive oxygen/nitrogen species metabolism is involved in the tolerant response of the invasive plant species *Ailanthus altissima* to drought and salinity. Environ Exp Bot 97:1–10

Flohe L, Günzler WA (1984) Assays of glutathione peroxidase. Method Enzyme 105:114–120

Gharsallah C, Fakhfakh H, Grubb D, Gorsane F (2016) Effect of salt stress on ion concentration, proline content, antioxidant enzyme activities and gene expression in tomato cultivars. AoB Plants 8: 12–25

Gill SS, Tuteja N (2010) Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiol Biochem 48(12):909–930

Giusti MM, Wrolstad RE (2001) Characterization and measurement of anthocyanins by UV-visible spectroscopy. Curr Protocols Food Chem 2:1–13

Gohari G, Alavi Z, Esfandiari E, Panahirad S, Hajihoseinlou S, Fotopoulos V (2020a) Interaction between hydrogen peroxide and sodium nitroprusside following chemical priming of *Ocimum basilicum* L. against salt stress. Physiol Plant 168(2):361–373

Gohari G, Mohammadi A, Akbari A, Panahirad S, Dadpour MR, Fotopoulos V, Kimura S (2020b) Titanium dioxide nanoparticles (TiO_2 NPs) promote growth and ameliorate salinity stress effects on essential oil profile and biochemical attributes of *Dracocephalum moldavica*. Sci Rep 10(1):1–14

Gohari G, Safai F, Panahirad S, Akbari A, Rasouli F, Dadpour MR, Fotopoulos V (2020c) Modified multiwall carbon nanotubes display either phytotoxic or growth promoting and stress protecting activity in *Ocimum basilicum* L. in a concentration-depended manner. Chemosphere 126:171

Hasanuzzaman M, Bhuyan MHM, Zulfiqar F, Raza A, Mohsin SM, Mahmud JA, Futija M, Fotopoulos V (2020) Reactive oxygen species and antioxidant defense in plants under abiotic stress: revisiting the crucial role of a universal defense regulator. Antioxidants 9(8): 681

Hatami M (2017) Toxicity assessment of multi-walled carbon nanotubes on *Cucurbita pepo* L. under well-watered and water stressed conditions. Ecotoxic Environ Safety 142:274–283

Hayat S, Hayat Q, Alyemeni MN, Wani AS, Pichtel J, Ahmad A (2012) Role of proline under changing environments: a review. Plant Signal Behav 7(11):1456–1466

Hoque MA, Banu MNA, Okuma E, Amako K, Nakamura Y, Shimoishi Y, Murata Y (2007) Exogenous proline and glycinebetaine increase NaCl-induced ascorbate–glutathione cycle enzyme activities, and proline improves salt tolerance more than glycinebetaine in tobacco Bright Yellow-2 suspension-cultured cells. J Plant Physiol 164(11): 1457–1468

Hu X, Kang J, Lu K, Zhou R, Mu L, Zhou Q (2014) Graphene oxide amplifies the phytotoxicity of arsenic in wheat. Sci Rep 4:6122

Ioannou A, Gohari G, Papaphilippou P, Panahirad S, Akbari A, Dadpour MR, Krasia-Christoforou T, Fotopoulos V (2020) Advanced nanomaterials in agriculture under a changing climate: the way to the future? Environ Exp Bot 176:104048

Kaya C, Tuna AL, Ashraf M, Altunlu H (2007) Improved salt tolerance of melon (*Cucumis melo* L.) by the addition of proline and potassium nitrate. Environ Exp Bot 60(3):397–403

Khan MN, Mobin M, Abbas ZK, AlMutairi KA, Siddiqui ZH (2017) Role of nanomaterials in plants under challenging environments. Plant Physiol Biochem 110:194–209

Khan I, Raza MA, Awan SA, Shah GA, Rizwan M, Ali B, Tariq R, Hassan MJ, Alyemeni MN, Brestic M, Zhang X (2020) Amelioration of salt induced toxicity in pearl millet by seed priming with silver nanoparticles (AgNPs): the oxidative damage, antioxidant enzymes and ions uptake are major determinants of salt tolerant capacity. Plant Physiol Biochem 156:221–232

Khanna-Chopra R, Semwal VK, Lakra N, Pareek A (2019) Proline–a key regulator conferring plant tolerance to salinity and drought. In: Plant tolerance to environmental stress: role of phytoprotectants. CRC Press, Boca Raton, pp 59–80

Kovinich N, Kayanja G, Chanoca A, Otegui MS, Grotewold E (2015) Abiotic stresses induce different localizations of anthocyanins in Arabidopsis. Plant Signal Behav 10(7):e1027850

Kubala S, Wojtyla Ł, Quinet M, Lechowska K, Lutts S, Garnczarska M (2015) Enhanced expression of the proline synthesis gene P5CSA in relation to seed osmopriming improvement of *Brassica napus* germination under salinity stress. J Plant Physiol 183:1–12

Li W, Tailhades J, O'Brien-Simpson NM, Separovic F, Otvos L, Hossain MA, Wade JD (2014) Proline-rich antimicrobial peptides: potential therapeutics against antibiotic-resistant bacteria. Amino Acids 46(10):2287–2294

Li H, Huang J, Lu F, Liu Y, Song Y, Sun Y, Lifshitz Y (2018) Impacts of carbon dots on rice plants: boosting the growth and improving the disease resistance. ACS Appl Bio Mater 1(3):663–672

Lim JH, Park KJ, Kim BK, Jeong JW, Kim HJ (2012) Effect of salinity stress on phenolic compounds and carotenoids in buckwheat (*Fagopyrum esculentum* M.) sprout. Food Chem 135(3):1065–1070

Lim SY, Shen W, Gao Z (2015) Carbon quantum dots and their applications. Chem Soc Rev 44(1):362–381

Lutts S, Kinet JM, Bouharmont J (1995) Changes in plant response to NaCl during development of rice (*Oryza sativa* L.) varieties differing in salinity resistance. J Exp Bot 46:1843–1852

Mane AV, Karadge BA, Samant JS (2010) Salinity induced changes in photosynthetic pigments and polyphenols of *Cymbopogon nardus* (L.) Rendle. J Chem Pharm Res 2(3):338–347

Maxwell K, Johnson GN (2000) Chlorophyll fluorescence-a practical guide. J Exp Bot 51(345):659–668

Molassiotis A, Fotopoulos V (2011) Oxidative and nitrosative signaling in plants: two branches in the same tree? Plant Signal Behav 6:210–214

Nakano Y, Asada K (1981) Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. Plant Cell Physiol 22(5):867–880

Omoto E, Kawasaki M, Taniguchi M, Miyake H (2009) Salinity induces granal development in bundle sheath chloroplasts of NADP-malic enzyme type C4 plants. Plant Prod Sci 12:199–207

Ozden M, Demirel U, Kahraman A (2009) Effects of proline on antioxidant system in leaves of grapevine (*Vitis vinifera* L.) exposed to oxidative stress by H_2O_2. Sci Hortic 119(2):163–168

Perveen SHAGUFTA, Nazir M (2018) Proline treatment induces salt stress tolerance in maize (*Zea mays* L. CV. Safaid Afgoi). Pak J Bot 50(4):1265–1271

Pollastri S, Savvides A, Pesando M, Lumini E, Volpe MG, Ozudogru EA, Faccio A, De Cunzo F, Michelozzi M, Lambardi M, Fotopoulos V, Loretto F, Centritto M, Balestrini R (2018) Impact of two arbuscular mycorrhizal fungi on *Arundo donax* L. response to salt stress. Planta 247(3):573–585

Quan LJ, Zhang B, Shi WW, Li HY (2008) Hydrogen peroxide in plants: a versatile molecule of the reactive oxygen species network. J Integr Plant Biol 50(1):2–18

Rao S, Shekhawat GS (2016) Phytotoxicity and oxidative stress perspective of two selected nanoparticles in *Brassica juncea*. Biotechnology 6(2):244

Reddy PS, Jogeswar G, Rasineni GK, Maheswari M, Reddy AR, Varshney RK, Kishor PK (2015) Proline over-accumulation alleviates salt stress and protects photosynthetic and antioxidant enzyme activities in transgenic sorghum [*Sorghum bicolor* (L.) Moench]. Plant Physiol Biochem 94:104–113

Sharma P, Jha AB, Dubey R, Pessarkli M (2012a) Reactive oxygen species, oxidative da mage, and antioxidative defense mechanism in plants under stressful conditions. Aust J Bot 1:1–26

Sharma P, Bhatt D, Zaidi MG, Saradhi PP, Khanna PK (2012b) Silver nanoparticle-mediated enhancement in growth and antioxidant status of *Brassica juncea*. Appl Biochem Biotechnol 167:2225–2233

Sinha S, Saxena R, Singh S (2005) Chromium induced lipid peroxidation in the plants of *Pistia stratiotes* L.: role of antioxidants and antioxidant enzymes. Chemosphere 58:595–604

Stewart RRC, Bewley JD (1980) Lipid peroxidation associated with accelerated aging of soybean axes. Plant Physiol 65:245–258

Tang W, Newton RJ (2005) Peroxidase and catalase activities are involved in direct adventitious shoot formation induced by thidiazuron in eastern white pine (*Pinus strobes* L.) zygotic embryos. Plant Physiol Biochem 43:760–769

Tang X, Mu X, Shao H (2015) Global plant-responding mechanisms to salt stress: physiological and molecular levels and implications in biotechnology. Crit Rev Biotechnol 35:425–437

Wang H, Zhang M, Song Y, Li H, Huang H, Shao M, Kang Z (2018) Carbon dots promote the growth and photosynthesis of mung bean sprouts. Carbon 136:94–102

Wani SH, Kumar V, Khare T, Guddimalli R, Parveda M, Solymosi K, Suprasanna P, Kishor PBK (2020) Engineering salinity tolerance in plants: progress and prospects. Planta 251:76

Xu C, Zhang Y, Cao L, Lu J (2010) Phenolic compounds and antioxidant properties of different grape cultivars grown in China. Food Chem 119:1557–1565

Yan Z, Guo S, Shu S, Sun J, Tezuka T (2011) Effects of proline on photosynthesis, root reactive oxygen species (ROS) metabolism in two melon cultivars (*Cucumis melo* L.) under NaCl stress. Afr J Biotechnol 10(80):18381–18390

Yan K, Shao H, Shao C, Chen P, Zhao S, Brestic M, Chen X (2013) Physiological adaptive mechanisms of plants grown in saline soil and implications for sustainable saline agriculture in coastal zone. Acta Physiol Plant 35(10):2867–2878

Yi Z, Li S, Liang Y, Zhao H, Hou L, Yu S, Ahammed GJ (2018) Effects of exogenous spermidine and elevated CO_2 on physiological and biochemical changes in tomato plants under iso-osmotic salt stress. J Plant Growth Regul 37(4):1222–1234

Zhang M, Gao B, Chen J, Li YC (2015) Effects of graphene on seed germination and seedling growth. J Nanopart Res 17(2):78

Zouari M, Ahmed CB, Elloumi N, Bellassoued K, Delmail D, Labrousse P, Rouina BB (2016) Impact of proline application on cadmium accumulation, mineral nutrition and enzymatic antioxidant defense system of *Olea europaea* L. cv Chemlali exposed to cadmium stress. Ecotoxic Environ Safety 128:195–205

Zouari M, Elloumi N, Labrousse P, Rouina BB, Abdallah FB, Ahmed CB (2018) Olive trees response to lead stress: exogenous proline provided better tolerance than glycine betaine. South Afric J Bot 118: 158–165