



# Salinity mitigates cadmium-induced phytotoxicity in quinoa (*Chenopodium quinoa* Willd.) by limiting the Cd uptake and improved responses to oxidative stress: implications for phytoremediation

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**Abstract** Cadmium (Cd) contamination and soil salinity are the main environmental issues reducing crop productivity. This study aimed to examine the combined effects of salinity (NaCl) and Cd on the physiological and biochemical attributes of quinoa (*Chenopodium quinoa* Willd.). For this purpose, 30-day-old plants of quinoa genotype “Puno” were transplanted in Hoagland’s nutrient solution containing diverse concentrations of Cd: 0, 50, 100, 200  $\mu\text{M}$  Cd, and salinity: 0, 150, and 300 mM NaCl. Results demonstrated that plant growth, stomatal conductance, and pigment contents were significantly lower at all Cd concentrations than the control plants. Quinoa plants exhibited improved growth and tolerance against Cd when grown at a lower level of salinity (150 mM NaCl) combined with Cd. In contrast, the elevated concentration of salinity (300 mM NaCl) combined with Cd reduced shoot and root growth of

experimental plants more than 50%. Combined application of salinity and Cd increased Na (25-fold), while lessened the Cd (twofold) and K (1.5-fold) uptake. A blend of high concentrations of Na and Cd caused overproduction of  $\text{H}_2\text{O}_2$  (eightfold higher than control) contents and triggered lipid peroxidation. The activities of antioxidant enzymes: ascorbate peroxidase (APX), catalase (CAT), peroxidase (POD), and superoxide dismutase (SOD) were 13, 12, 7 and ninefold higher than control to mitigate the oxidative stress. Due to restricted root to shoot translocation, and greater tolerance potential against Cd, the quinoa genotype, Puno, is suitable for phytostabilization of Cd in saline soils.

**Keywords** Cadmium · Antioxidants · Salt stress · ROS · Phytostabilization

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## Introduction

Cadmium (Cd) contamination in soil has become a challenging issue and is primarily contributed by anthropogenic activities, including agricultural practices, mining activities, industrial effluents, and wastewater irrigation (Amjad et al., 2021; Anwar et al., 2021). Since the Cd is not involved in any known biological functions in plants, it is recognized as a non-essential element (Rehman et al., 2019). The concentration of this non-essential heavy metal in the soil varies considerably, and it has been ranked fourth in the list of highly toxic elements for plant growth (Qayyum et al., 2017).

Due to the toxic nature of Cd and being a non-essential element, its uptake, even in traces, causes a wide range of impairments to plant biochemical, physiological, and morphological functions (Zhang et al., 2020). Harmful effects of Cd may compromise plant growth and biomass, damage to chlorophyll, rupturing of cellular membranes, and reduction in nutrient uptake capacity (Amjad et al., 2021; Shanying et al., 2017). Likewise, Cd toxicity causes overproduction of reactive oxygen species (ROS), which are incredibly detrimental to plant tissues. Cd-induced toxicity and oxidative stress triggered by ROS in plants are well documented (Rehman et al., 2019). Oxidative stress is caused by various ROS including hydrogen peroxide ( $H_2O_2$ ), superoxide ( $O_2^{\cdot-}$ ), hydroxyl ( $HO^{\cdot}$ ) radicals, and singlet oxygen ( $^1O_2$ ), imparting severe damages to carbohydrates, lipids, proteins and nucleic acids (Anwar et al., 2021; Rehman et al., 2019). The implications of these ROS are mitigated by boosting the activities of antioxidant enzymes such as ascorbate peroxidase (APX), catalase (CAT), peroxidase (POD), and superoxide dismutase (SOD) (Amjad et al., 2021; Souri et al., 2018).

Soil salinity is another serious menace for crop production worldwide, which has affected about 6% of the global land including 20% of areas that have become salinized due to irrigation with salt contaminated groundwater (Abbas et al., 2021; Qadir et al., 2014). Salinity prompts various adverse effects on plants, including osmotic stress, reduced gas exchange, ionic imbalance, chlorophyll concentration, and relative water contents in leaf (Flowers et al., 2015; Hu et al., 2017). These impairments consequently cause a reduction in biomass and grain yield of crops (Abbas et al., 2018). However, the response of

plants to salt stress varies with the type of salt-affected soils and plant genotypes (Abbas et al., 2021).

The efficient use of salt-affected soils and waters has become vital for feeding the country's rapidly growing population. Compared to glycophytic crops such as wheat and rice, halophytic grain crops suitable for saline soils need to be incorporated into our cropping system (Shabala et al., 2013). Quinoa (*Chenopodium quinoa* Willd.) cultivation is regarded as the most appropriate way for sustainable utilization of salt-affected soils (Abbas et al., 2021; Jacobsen et al., 2009). Quinoa is a potential food security crop because of its high nutritional quality grains, and it can be successfully grown on compromised soils (Abbas et al., 2021; Riaz et al., 2020). It is highly suitable for cultivating on metal-contaminated saline soils due to its potential to stabilize the metals within the soil (Amjad et al., 2021; Iftikhar et al., 2021; Parvez et al., 2020). However, to the best of our knowledge, the growth and physiological responses of quinoa genotypes under combined effects of Cd and salinity have not been explored so far, perhaps due to complicated interactions between Na and Cd. There are few contradictory reports regarding this complex interaction. For instance, Rehman et al. (2019) observed increased Cd uptake, while others (Cheng et al., 2018; Mariem et al., 2014) noticed reduced Cd uptake by plants when Cd and salinity were applied together. Hence, the present study was designed with trifold objectives: firstly, to analyze the growth and physiological responses of quinoa genotype "Puno" exposed to the combined application of Cd and salinity; secondly, to evaluate the effects of salinity on the Cd tolerance and phytoremediation potential of quinoa; lastly, to explore the tolerance mechanisms of quinoa against the combined stress of Cd and salinity.

## Materials and methods

### Experimental description

An experiment based on solution culture was carried out at COMSATS University Islamabad, Vehari Campus (30. 231°N, 72. 211°E) during 2020–2021. The temperature during the study ranged from 10–26 °C. Quinoa genotype "Puno" was used in this experiment because of its better adaptation to salinity and heavy metals (Iftikhar et al., 2021). Thirty-day-old

quinoa plants were transplanted to half-strength Hoagland's nutrient solution (Hoagland & Arnon, 1950) containing macro-nutrients in mM (Ca 2.5, Mg 1.0, S 1.0, K 3.0, N 3.75, P 0.5) and micro-nutrients in  $\mu\text{M}$  (Fe 26.0, Cu 0.16, Mn 4.55, Zn 0.38, B 23.15, Mo 0.25, Cl 9.0). Plant samples were allowed to acclimatize for one week in the solution before applying Cd and salinity treatments. The salts of cadmium chloride ( $\text{CdCl}_2$ ) and sodium chloride ( $\text{NaCl}$ ) were used in the solution culture to obtain the final concentration of 0, 50, 100, 200  $\mu\text{M}$  Cd, and 0, 150 300 mM of salinity, respectively. Salinity treatments were applied in two splits, whereby the first half was used at the time of the Cd application, while the remaining half was applied two days thereafter. All the treatments were applied alone as well as in combinations. The nutrient solution was replaced every week and with new solution, Cd and salinity treatments were applied as a single dose. The pH was maintained at  $6.5 \pm 0.2$  using 0.1 N HCl or NaOH. Each treatment contained four replicates, with one plant for each replication.

#### Harvesting and data collection

Four weeks after transplanting in the solution, plants were harvested where the roots were separated from the shoots and washed in 0.01 M HCl solution, followed by rinsing in distilled water to remove the adsorbed Cd as detailed by Iftikhar et al. (2021). Lengths of root/shoot were recorded. Afterward, plant materials were air-dried, followed by oven drying at 70 °C for 48 h, and dry weights were noted.

#### Digestion of plant material and analyses

The roots and shoots of the experimental plants were ground separately using a porcelain grinder. Both the roots and shoots were separately digested using a blend of  $\text{HClO}_4$  and  $\text{HNO}_3$  at 1:2 ratio, respectively. Thereafter, each sample was diluted with the distilled water up to 50 mL volume. Sodium (Na) and potassium (K) ions' concentrations were estimated on a flame photometer (BWB-XP5). However, the Cd concentration was measured on an atomic absorption spectrometer (PerkinElmer Model: Pin AAcle 900F, Inc. USA) using internal standards (CPAchem, Bulgaria) while standardizing the equipment with certified reference material (SRM 1547, peach leaves).

Bioconcentration factor, translocation factor, and tolerance index

Bioconcentration (BCF) and translocation factor (TF) were calculated as described by Rehman et al. (2019). The concentration ratio of Cd in plants and solution was expressed as BCF, whereas the concentration ratio of Cd between root and shoot was denoted as TF. The tolerance index (TI) was calculated by dividing the dry weights of the Cd stressed plant by the dry weights of control plants.

#### Leaf pigments and stomatal conductance

All types of chlorophyll, chlorophyll a, chlorophyll b, and total chlorophyll values, were assessed by following the procedure outlined by Lichtenthaler (1987). For this analysis, leaf samples (1.0 g) were dipped for few seconds in liquid nitrogen to cease the metabolic processes and ground in 80% hydro-acetone solution. After grinding, the samples were centrifuged at  $3000 \times g$  for 10 min and the supernatant was collected. The absorbance of collected supernatant was recorded by UV–Vis spectrophotometer (Lambda 25, PerkinElmer, Inc. USA) at 663.2 and 646.8 nm wavelengths. Stomatal conductance of fully grown second leaf from the top was measured with a portable leaf porometer (Decagon Devices, Pullman, WA, USA).

#### Membrane stability index (MSI)

The membrane stability index (MSI) of plant leaves was assessed by evaluating the electrical conductance (EC) of the leaf leachate produced in distilled water as detailed by Rehman et al. (2019). The EC was measured at two different time intervals (10 and 30 min.), and two different temperature regimes (40 °C for EC1 and 100 °C for EC2), respectively. The MSI values were calculated as per Eq. 1 given below:

$$\text{MSI} = 1 - \left( \frac{\text{EC1}}{\text{EC2}} \right) \times 100 \quad (1)$$

## Hydrogen peroxide and lipid peroxidation

Hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) and lipid peroxidation (ROS) determinations were carried out by using the method of Islam et al. (2008). Briefly, 0.5 g leaf samples were ground/homogenized in 0.1% trichloroacetic acid and centrifuged at  $12,000 \times g$  for 20 min. One mL (1 mL) of supernatant was taken, and a buffer solution containing 1 mL each of potassium iodide (2 M) and potassium phosphate (10 mM) was added into it while maintaining the pH at 7.0. The absorbance of the reaction mixture was noted at 390 nm wavelength using UV–Vis spectrophotometer, and the concentration of  $\text{H}_2\text{O}_2$  in the sample was calculated. Assays for lipid peroxidation were carried out by determining the thiobarbituric acid reactive substances (TBARS) in the sample according to the method devised by Hodges et al. (1999).

## Enzymatic activities

The most newly appeared leaves of quinoa plants were used for assessing the activities of antioxidant enzymes; APX, CAT, POD, and SOD. For these assays, 0.5 g leaf samples were ground in 0.1 M phosphate buffer (pH 7.0) under liquid nitrogen. The ground samples were centrifuged at  $15,000 \times g$  for 30 min at 4 °C. The enzymatic activity of SOD was estimated based on a 50% reduction of nitro blue tetrazolium (NBT) according to the methods devised by Dhindsa et al. (1981). At the same time, the activity of APX was measured by following the procedures of Nakano and Asada (1981) and considered as 1 M ascorbate  $\text{min}^{-1} \text{mg}^{-1}$  protein. The enzymatic activity of CAT in the leaf sample was assessed as explained by Aebi (1984) and considered 1 M of  $\text{H}_2\text{O}_2$  degraded  $\text{min}^{-1} \text{mg}^{-1}$  protein. The enzymatic activity of POD was determined as 1 M of guaiacol that oxidized  $\text{min}^{-1} \text{mg}^{-1}$  protein (Hemeda and Klein 1990).

## Statistical analyses

A completely randomized design (CRD) was used in the present study. The data were statistically analyzed using one-way analysis of variance using “Statistix 8.1”. The least significant difference test at a 5% probability level was used for treatment comparison (Steel et al., 1997).

## Results

### Plant growth

Results demonstrated that root and shoot growth of quinoa genotype “Puno” decreased with the increasing concentration of Cd in the growth medium (Table 1). However, co-application of salinity (150 mM) with various concentrations of Cd enhanced plant growth compared to Cd stressed plants. High doses of salinity and Cd negatively impacted plant growth. The plants exposed to the highest salinity level (300 mM) and Cd concentration showed a remarkable reduction in plant growth. Markedly, the root and shoot lengths were decreased by 55 and 58%, respectively, when plants were grown at the sole treatment of Cd (200  $\mu\text{M}$ ). Contrary to these observations, respective reductions in root and shoot were noted as 46 and 47% when plants were supplied with a mild salinity level (150 mM), at the same Cd concentration of 200  $\mu\text{M}$ . Co-application of the highest concentration of Cd (200  $\mu\text{M}$ ) and salinity (300 mM) exerted detrimental effects on plant growth with a significant reduction of 64 and 57% in the root and shoot length of quinoa plants, respectively. The dry weights of roots and shoots exhibited a similar trend at higher doses of Cd and salinity. Mild application of salinity at a lower concentration of Cd had a soothing effect on plant growth. It caused a lesser reduction of 43 and 45% in the dry weights of root and shoot, respectively, as compared to the control treatment. In comparison, decreases in dry weights of these plant parts were noted as 57 and 56%, respectively, when plants were grown at higher doses of Cd (200  $\mu\text{M}$ ) and salinity (300 mM).

### Chlorophyll contents and stomatal conductance

As evident from the data in Table 2, chlorophyll contents of quinoa leaves were negatively influenced at higher doses of Cd and salinity. Except for none, all types of green pigment (chlorophyll a, chlorophyll b, total chlorophyll) and stomatal conductance of quinoa decreased at greater salinity and Cd concentration in the nutrient solution. Opposing these results, plants exposed to lower salinity levels and Cd had a higher concentration of chlorophyll contents and improved stomatal conductance. Increasing Cd level to 200  $\mu\text{M}$  caused respective reductions of 30, 50, 38, and 51% in

**Table 1** Effect of salinity (mM) and Cd (μM) on the agronomic traits of quinoa plants growing at different levels of salinity and Cd under hydroponics

Treatments	RL (cm)	SL (cm)	RDW (g plant <sup>-1</sup> )	SDW (g plant <sup>-1</sup> )
Control	22.5 ± 0.9 a	22.3 ± 0.7 a	0.3 ± 0.01 a	0.4 ± 0.02 a
S 150	19 ± 0.8 b	19 ± 0.5 b	0.26 ± 0.005 b	0.35 ± 0.014 b
S 300	16 ± 0.4 cd	15 ± 0.3 d	0.22 ± 0.010 de	0.29 ± 0.012 c
Cd 50	15.5 ± 0.6 d	15.3 ± 0.1 d	0.25 ± 0.006 c	0.30 ± 0.011 c
Cd 50 S 150	17 ± 0.7 c	17 ± 0.6 c	0.28 ± 0.003 b	0.35 ± 0.011 b
Cd 50 S 300	12.2 ± 0.7 e	13 ± 0.8 e	0.23 ± 0.004 d	0.28 ± 0.013 c
Cd 100	13.8 ± 0.4 de	12.8 ± 0.5 e	0.21 ± 0.007 e	0.24 ± 0.009 d
Cd 100 S 150	15.5 ± 0.2 c	15 ± 0.8 d	0.23 ± 0.005 d	0.24 ± 0.010 d
Cd 100 S 300	11.5 ± 0.8 e	10.4 ± 0.2 f	0.18 ± 0.005 f	0.18 ± 0.010 f
Cd 200	9.5 ± 0.5 f	10 ± 0.5 f	0.15 ± 0.003 g	0.18 ± 0.013 f
Cd 200 S 150	11.9 ± 0.5 e	12 ± 0.5 e	0.17 ± 0.006 f	0.22 ± 0.009 e
Cd 200 S 300	8.0 ± 0.4 g	8.0 ± 0.3 g	0.13 ± 0.009 h	0.15 ± 0.009 g

Data are mean of four replications ± SE. For each parameter, values with different letter(s) are significantly different from each other at 5% significance level

S salinity (NaCl), RL root length, SL shoot length, RDW root dry weight, SDW shoot dry weight

chlorophyll a, chlorophyll b, total chlorophyll, and stomatal conductance. When a higher concentration of Cd was augmented with salinity, the corresponding reduction in these traits of quinoa plants was 27, 40, 32, and 43%, respectively, compared to control plants. Reductions in the physiological attributes of experimental plants were more pronounced at elevated levels of Cd and salinity (Table 2).

**Ionic contents in quinoa**

Increasing levels of NaCl in the nutrient solution caused increased accumulation of Na ions both in the below- and aboveground parts of quinoa plants and vice versa (Fig. 1a, b). The combined application of salinity and Cd increased Na ions concentration in roots and shoots of the experimental plants as compared to plants exposed to salinity treatment only. Although mild salinity treatment did not influence the K concentration in quinoa plants (Fig. 1c, d), combined salinity and Cd concentration reduced K ions in the root and shoot of quinoa compared to control treatment.

**Metal accumulation and tolerance**

Accumulation of Cd in the plant parts was positively correlated with metal application in the growth medium. The increasing concentration of Cd in the nutrient solution caused a more significant accumulation of Cd in the roots and shoots of quinoa plants (Table 3). The combined application of salinity and Cd treatments decreased Cd contents in the roots and shoots of quinoa. Still, overall, a greater concentration of metal was reported in roots as compared to shoots. The BCF was higher in plants that received Cd treatments than those treated only with Cd and salinity. Application of salinity along with Cd reduced the BCF value; however, the value of TF was always < 1 regardless of the sole application of Cd or combined with salinity (Table 3). The TI was lessened with increasing levels of salinity and Cd in the growth medium and vice versa. The joint application of mild level of salinity with Cd increased TI of quinoa against Cd stress (Table 3).

**Oxidative stress**

Increasing concentration of Cd imparted oxidative stress in the experimental plants by raising the H<sub>2</sub>O<sub>2</sub> and TBARS levels, as shown in Figs. 2a, b. The

**Table 2** Effect of salinity (mM) and Cd ( $\mu\text{M}$ ) on the physiological attributes of quinoa plants treated with different concentrations of salinity and Cd under hydroponics

Treatments	Chl a ( $\mu\text{g g}^{-1}$ FW)	Chl b ( $\mu\text{g g}^{-1}$ FW)	T. Chl ( $\mu\text{g g}^{-1}$ FW)	gs ( $\text{mmol m}^{-2} \text{s}^{-1}$ )
Control	260 $\pm$ 5 a	160 $\pm$ 4 a	420 $\pm$ 8 a	490 $\pm$ 15 a
S 150	255 $\pm$ 7 a	155 $\pm$ 3 a	410 $\pm$ 7 a	460 $\pm$ 7 b
S 300	220 $\pm$ 8 c	120 $\pm$ 5 c	340 $\pm$ 10 c	390 $\pm$ 10 d
Cd 50	220 $\pm$ 5 c	115 $\pm$ 3 cd	335 $\pm$ 8 cd	390 $\pm$ 8 d
Cd 50 S 150	230 $\pm$ 3 b	130 $\pm$ 2 b	360 $\pm$ 5 b	430 $\pm$ 5 c
Cd 50 S 300	180 $\pm$ 2 f	100 $\pm$ 2 e	280 $\pm$ 4 e	370 $\pm$ 9 e
Cd 100	205 $\pm$ 4 d	110 $\pm$ 4 d	315 $\pm$ 8 d	330 $\pm$ 20 f
Cd 100 S 150	235 $\pm$ 6 b	130 $\pm$ 3.5 b	365 $\pm$ 5 b	350 $\pm$ 5 ef
Cd 100 S 300	160 $\pm$ 8 f	65 $\pm$ 5 h	225 $\pm$ 8 g	240 $\pm$ 8 h
Cd 200	180 $\pm$ 10 ef	90 $\pm$ 4 f	270 $\pm$ 7 f	240 $\pm$ 17 h
Cd 200 S 150	190 $\pm$ 5 e	105 $\pm$ 5 d	295 $\pm$ 6 e	280 $\pm$ 6 g
Cd 200 S 300	140 $\pm$ 3 g	80 $\pm$ 3 g	230 $\pm$ 6 h	180 $\pm$ 6 i

The data are means of four replications  $\pm$  SE. For each parameter, values with different letter(s) are significantly different from each other at 5% significance level

S salinity (NaCl), Chl a chlorophyll a, Chl b chlorophyll b, T. Chl total chlorophyll, gs stomatal conductance

combined application of higher doses of salinity and Cd caused additional oxidative damage. Numerically, the higher Cd concentration in the nutrient solution resulted in a sixfold increase to  $\text{H}_2\text{O}_2$  and TBARS concentrations than control treatments. In contrast, respective increases in  $\text{H}_2\text{O}_2$  and TBARS were noted as 6.7- and 6.8-fold when mild salinity treatment was applied at the same concentration of Cd. However, increases in these stress attributes were reported to be  $> 8$  folds at the highest levels of salinity and Cd treatments compared to control. Membrane stability index (MSI) decreased as the salinity and Cd levels increased in the growth medium (Fig. 2c). Compared with control plants, individual applications of salinity and Cd, respectively, reduced the MSI to 1.1- and 1.8-fold. Still, the effect was multiplied (2.2 folds) when salinity and Cd were applied together to experimental plants.

#### Activities of antioxidant enzymes

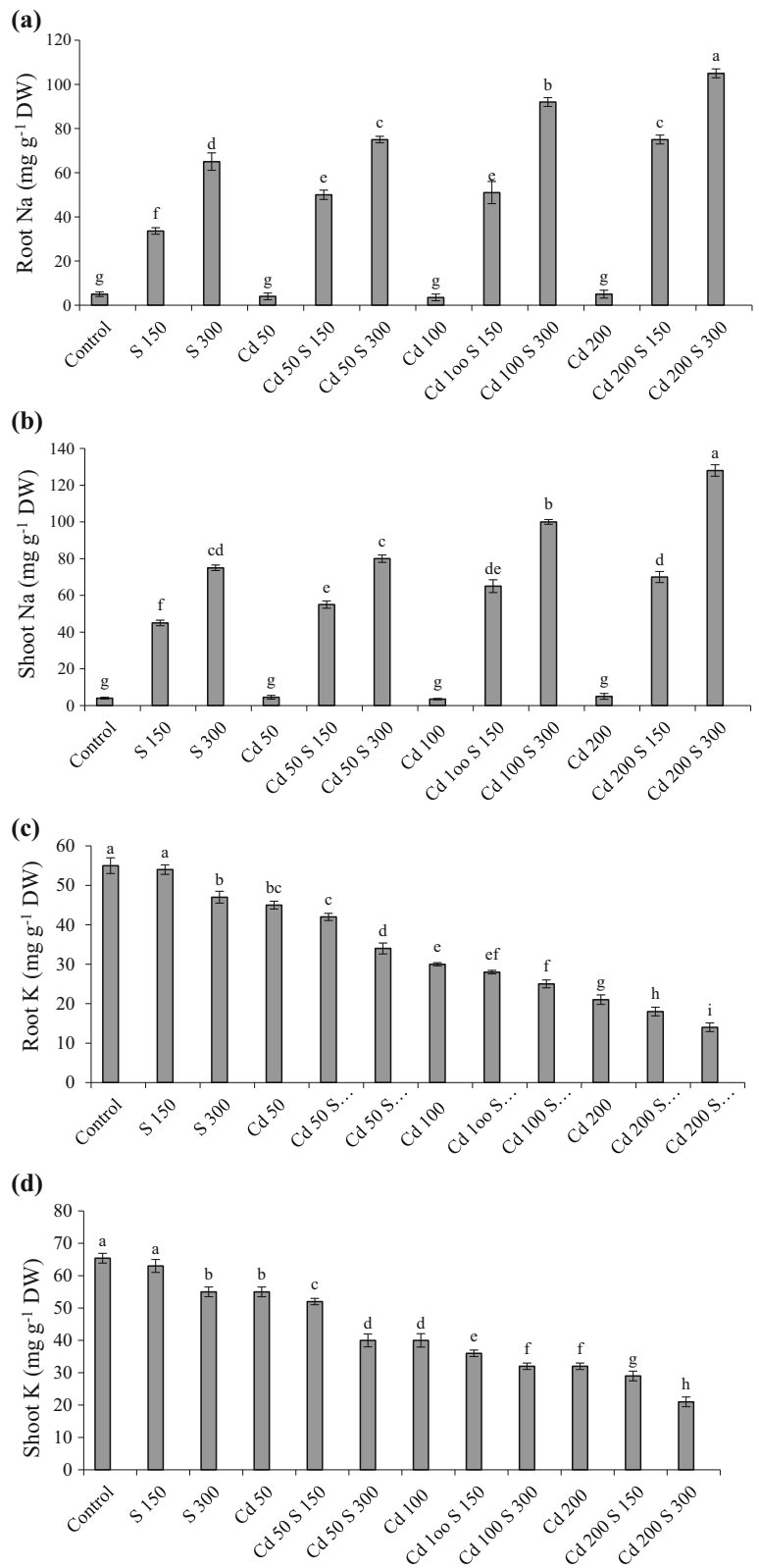
Activities of antioxidant enzymes; APX, CAT, POD and SOD revealed variable responses to salinity and Cd concentration in the growth medium (Fig. 3a–d). All these enzymes were overexpressed in quinoa plants, exposed to the combined application of higher doses of salinity and Cd. For instance, in plants treated

with 200  $\mu\text{M}$  Cd treatment, the respective increase in the aforementioned enzymes was 10.5, 7.2, 11, and 5.5 folds when compared to the control treatment. However, at lower salinity level, the respective activities of APX, CAT, POD, and SOD were only 3.3, 4, 2.5, and 3.6 folds higher with respect to the control plants. The combined treatment of higher quantities of salinity and Cd increased the activities of all these antioxidant enzymes by 12.5-, 9.2-, 13.3-, and 7.5-fold concerning control.

#### Discussion

The literature regarding the interaction of salinity and heavy metals is not conclusive due to contradictory outcomes. Few studies have reported that the uptake of heavy metals increases in the presence of salinity (Rehman et al., 2019; Shabir et al., 2018), while others have opposite view point (Cheng et al., 2018; Parvez et al., 2020). The results of the present study were quite variable depending on the levels of salinity and Cd concentration in the growth medium. For instance, the growth of quinoa plants was not greatly hampered up to the salinity level of 150 mM NaCl in the culture, highlighting the salt tolerance potential of this super-food plant (Iftikhar et al., 2021). Quinoa plants

**Fig. 1** Effect of salinity (mM) and Cd ( $\mu\text{M}$ ) on the concentration of Na in roots (a), shoots (b), and K contents in roots (c) and shoot (d) of quinoa plants exposed to different levels of salinity and Cd under hydroponics. The data represent the mean values of four replicates  $\pm$  SE. The values with different letter(s) are significantly different at 5% significance level



**Table 3** Effect of salinity (mM) and Cd ( $\mu\text{M}$ ) on metal accumulation, bioconcentration factor (BCF), translocation factor (TF) and tolerance index (TI) of quinoa plants exposed to different levels of salinity and Cd under hydroponics

Treatments	Cd conc. Root ( $\text{mg kg}^{-1}$ DW)	Cd conc. shoot ( $\text{mg kg}^{-1}$ DW)	BCF	TF	TI (%)
Control	$0.6 \pm 0.01$ f	$0.5 \pm 0.01$ g	–	$0.83 \pm 0.01$ a	–
S 150	$0.6 \pm 0.01$ f	$0.3 \pm 0.02$ g	–	$0.50 \pm 0.01$ b	$88 \pm 2.5$ a
S 300	$0.5 \pm 0.01$ f	$0.4 \pm 0.01$ g	–	$0.80 \pm 0.01$ a	$73 \pm 3.8$ bc
Cd 50	$40 \pm 0.5$ c	$20 \pm 1.1$ c	$3.55 \pm 0.2$ a	$0.50 \pm 0.02$ b	$75 \pm 2.5$ b
Cd 50 S 150	$28 \pm 1.3$ d	$13 \pm 1.0$ e	$2.31 \pm 0.13$ b	$0.46 \pm 0.13$ c	$85 \pm 4.1$ ab
Cd 50 S 300	$17 \pm 2.0$ e	$9.0 \pm 0.5$ f	$1.60 \pm 0.2$ c	$0.53 \pm .03$ b	$70 \pm 2.3$ c
Cd 100	$52 \pm 1.8$ b	$25.5 \pm 1.1$ b	$2.27 \pm 0.18$ b	$0.49 \pm 0.02$ bc	$60 \pm 3.9$ d
Cd 100 S 150	$33 \pm 1.1$ cd	$17 \pm 1.1$ d	$1.51 \pm 0.1$ c	$0.52 \pm 0.02$ b	$60 \pm 4.2$ d
Cd 100 S 300	$30 \pm 0.5$ d	$14 \pm 1.0$ e	$1.24 \pm 0.1$ e	$0.47 \pm 0.02$ c	$45 \pm 3.5$ f
Cd 200	$65 \pm 1.9$ a	$31.6 \pm 0.5$ a	$1.41 \pm 0.1$ d	$0.49 \pm 0.01$ bc	$45 \pm 3.9$ f
Cd 200 S 150	$40 \pm 2.0$ c	$20 \pm 0.5$ c	$0.89 \pm .06$ f	$0.50 \pm 0.06$ b	$55 \pm 3.2$ e
Cd 200 S 300	$30 \pm 1.5$ d	$16 \pm 1.0$ d	$0.71 \pm 0.04$ g	$0.53 \pm 0.04$ b	$38 \pm 2.9$ g

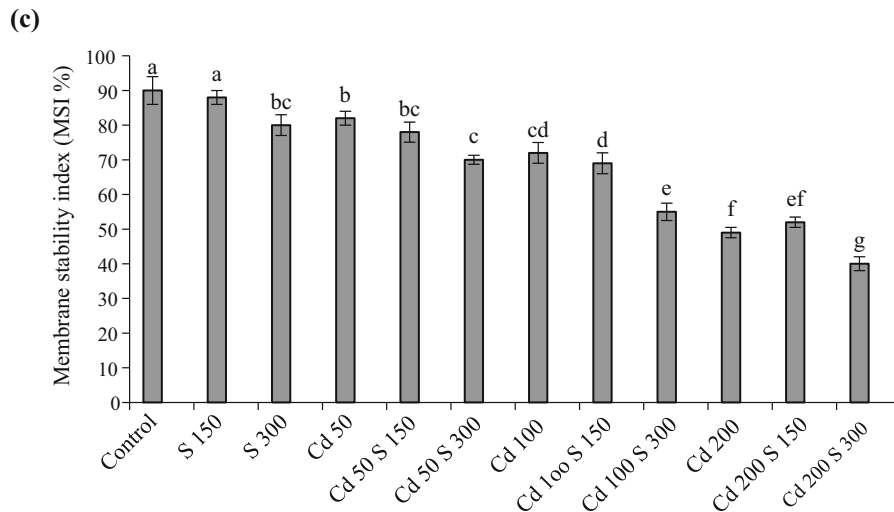
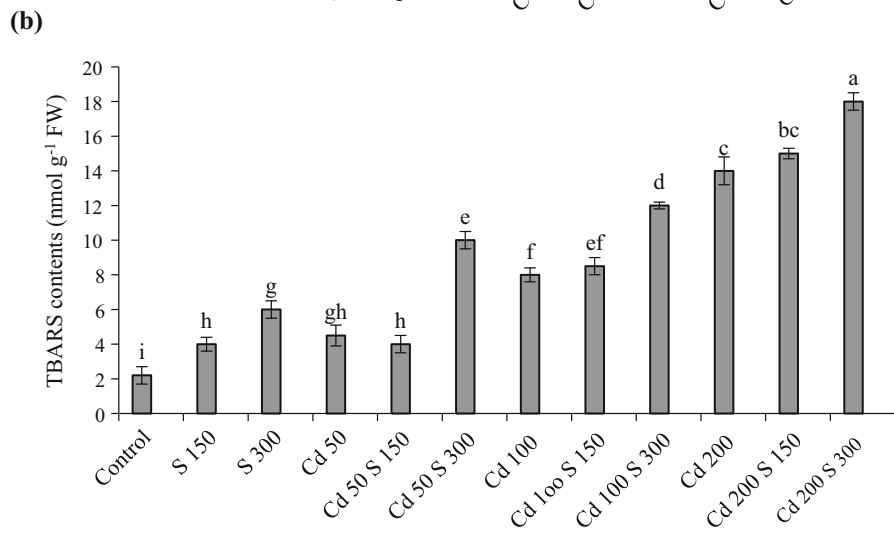
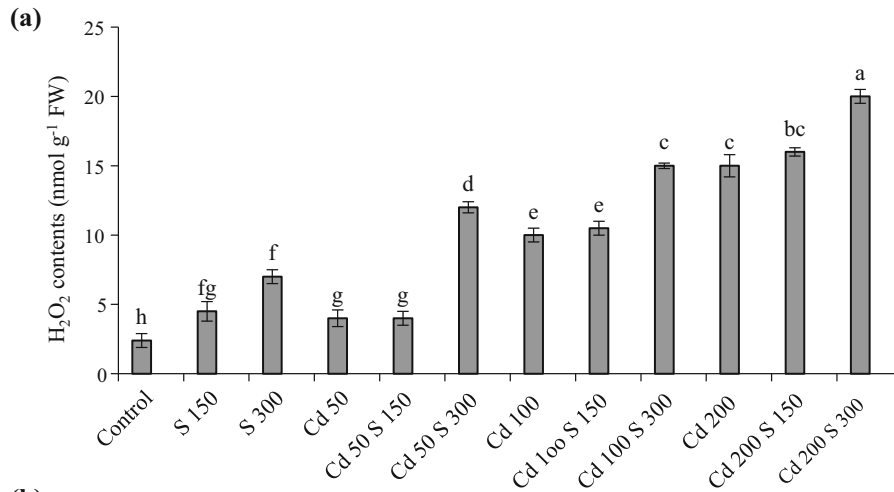
The data are means of four replications  $\pm$  SE. For each parameter, values with different letters are significantly different from each other at 5% significance level

exposed to a NaCl concentration of 300 mM showed significantly stunted growth, corroborating the previous research where elevated salinity levels (300 mM) impaired the growth and yield of quinoa plants (Iftikhar et al., 2021; Riaz et al., 2020). The stunted growth of quinoa could be ascribed to the salt-induced osmotic stress, ion toxicity, oxidative stress, and chlorophyll degradation (Abbas et al., 2021). Although redundant plant growth was noticed even at the lowest concentration of Cd (50  $\mu\text{M}$  Cd) in the medium, the higher level of Cd (200  $\mu\text{M}$ ) caused a remarkable decline in the biomass of quinoa plants. Previous research (e.g., Amjad et al. (2021) and the references therein) noted such harmful effects of Cd on the growth and yield of quinoa. This is not surprising, considering the non-essentiality of Cd in any physiological or metabolic processes of plants (Rehman et al., 2019). Instead, Cd accumulation within the plant body interferes with nutrients uptake and increases the toxicity of up taken ions, impairs photosynthetic apparatus, slows down the enzymatic activities, and halts the plant–water relations (Hodges et al., 1999; Shabir et al., 2018). These Cd-induced impairments might have caused the decline in the growth and yield of quinoa plants in the current study. The stressing effects of Cd on the experimental plants were lessened during co-application of salinity and Cd, perhaps because salinity improved the Cd

tolerance index (TI) of quinoa plants, which being halophytic grows successfully under saline conditions. Another reason for enhanced growth under co-application of Cd and salinity could be the limited uptake of Cd by quinoa under moderate salt stress levels. However, co-application of greater amounts of Cd and salinity did not improve plant growth and TI. Our observations are in line with others, where Iftikhar et al. (2021) found that salinity application eased up the quinoa plants to survive under Pb stress, which would otherwise die under the elevated concentration of non-essential heavy metal. Similarly, Cheng et al. (2018) observed an improved growth of the salt-tolerant plant, *Carpobrotus rossii*, when jointly treated with Cd and salinity; however, at higher levels of Cd and NaCl, plant growth was decreased. From the current study, it would not be unwise to conclude that a low level of salinity in the growth medium may prove beneficial for some halophytic plant species under heavy metal stress (Cheng et al., 2018; Iftikhar et al., 2021; Manousaki et al., 2009; Zhang et al., 2020).

Chlorophyll contents and stomatal conductance were also declined at higher concentrations of salinity and Cd. Our findings corroborate with other reports (Abbas et al., 2021; Chen et al., 2020; Rehman et al., 2019), where Cd and salinity applications hindered the leaf pigments and stomatal homeostasis. These anomalies can be attributed to the damage to





◀ **Fig. 2** Effect of salinity (mM) and Cd ( $\mu\text{M}$ ) on;  $\text{H}_2\text{O}_2$  (a), TBARS (b), and MSI (c) of quinoa plants provided with different concentrations of salinity and Cd under hydroponics. The data presented are the means of four replicates  $\pm$  SE. Values with different letter(s) are significantly different from each other at 5% significance level

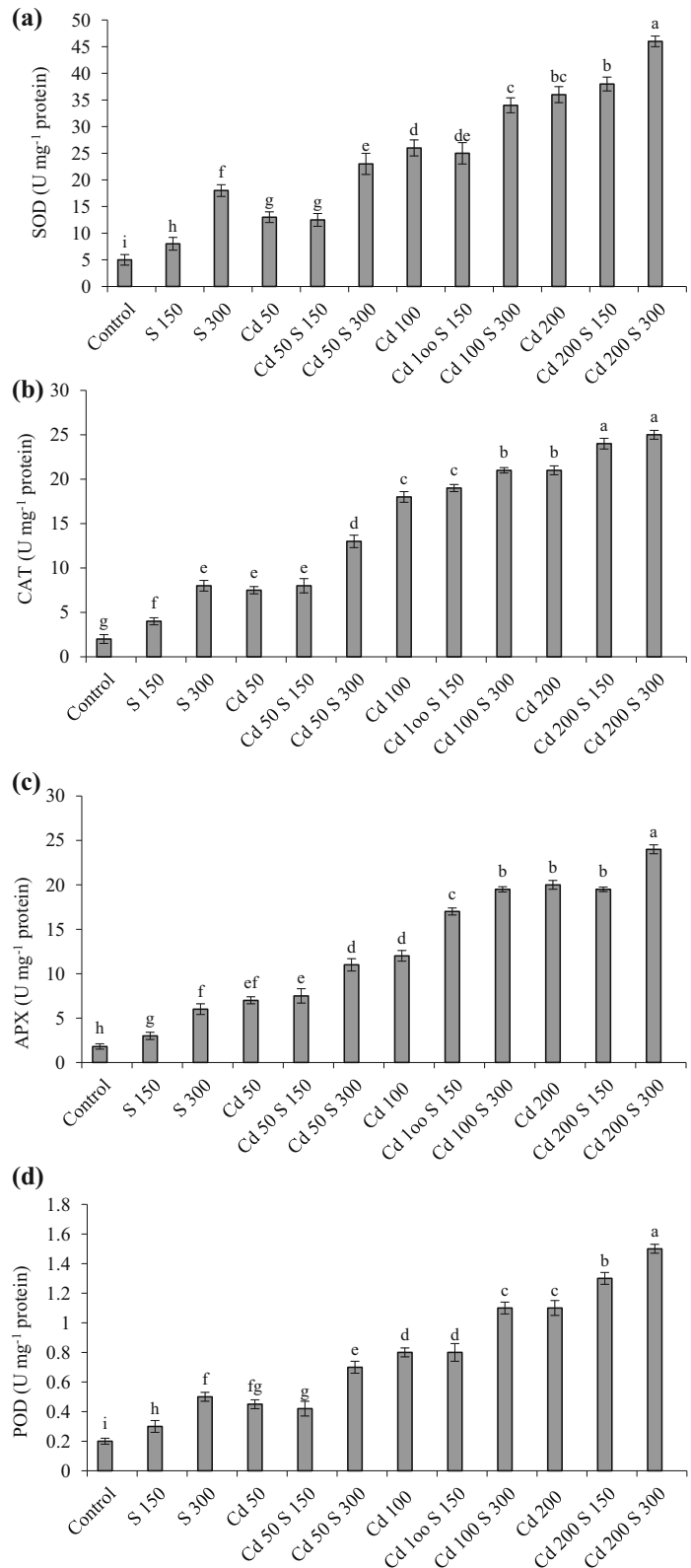
chloroplast membrane and disorder in thylakoids arrangement in photosynthetic apparatus, which are the known consequences of Cd accumulation in plants (Chen et al., 2020; Zhang et al., 2020). Moreover, chlorophyll molecules are damaged due to the toxicity induced by Cd and Na to the macronutrients (Riaz et al., 2020; Zhang et al., 2020). Reduction in stomatal conductance of leaves under Cd stress is ascribed to the non-stomatal limitation of photosynthesis, as noted by (Gallego et al., 2012). Mild application of salinity in combination with Cd improved the stomatal conductance and pigments (Zhang et al., 2020). As salinity positively influences the photosynthetic electron transport chain and maintains the ultrastructure integrity of chloroplasts, hence improving the chlorophyll contents and stomatal functioning (Wali et al., 2016). Moreover, a lower salinity level is well documented to enhance  $\text{CO}_2$  absorption per unit leaf area, leading to increased stomatal conductance (Iftikhar et al., 2021; Nawaz et al., 2016).

The accumulation of Na by quinoa was boosted with an increasing salt concentration in the nutrient solution, corroborating with the findings of Riaz et al. (2020) and (Iftikhar et al., 2021). Moreover, the growth and yield of the quinoa genotype are prone to high Na contents (Abbas et al., 2021). However, in the combined application of salinity and Cd, Na contents were higher both in the roots and aboveground foliage of plants compared to salinity alone treatments (Zhang et al., 2020). Halophytes, including quinoa, use different strategies to adapt to the medium containing an excessive amount of Na. For instance, some genotypes use an exclusion strategy, while others sequester Na in leaf vacuoles (Abbas et al., 2021; Shabala et al., 2013). In our study, quinoa genotype “Puno” accumulated more Na in shoot than root, indicating that vacuolar sequestration was the prominent mechanism of Na tolerance in this genotype (Iftikhar et al., 2021). A lower degree of salinity did not influence K uptake by the experimental plants either way. Still, increasing salinity levels and a

combination of salinity and Cd lessened the K concentration in plant tissues. Similar observations were reported by Iftikhar et al. (2021) while studying Pb and salinity interactions. Literature reveals that K, being an essential macronutrient, has many vital roles in plant metabolism, including osmotic adjustments, enzyme activation, cell enlargement, chlorophyll formation, cytoplasmic pH, and membrane stability (Munns & Tester, 2008). It is well documented that higher K contents in plants exposed to salinity stress usually have better salinity tolerance capacity (Abbas et al., 2021; Riaz et al., 2020). Both the Na and K ions exhibit similar hydration energy as well as ionic radii. Therefore, under salt stress, K channels are occupied by Na for entry into the cell (Marschner, 1995), and additional uptake of Na ions limits the movement of K ions leading to the deficiency of this essential nutrient (Abbas et al., 2021; Parvez et al., 2020). Likewise, the aggregation of Cd in plants also causes limited uptake of different elements, including K (Gallego et al., 2012; Rehman et al., 2019). This controlled uptake of K in the presence of Cd might be due to competition for the same cations’ channels (Amjad et al., 2021; Gallego et al., 2012). It has been reported that Cd interferes with the uptake of K at the root level and its transportation within the cell (Rehman et al., 2019). The limited uptake of K results in malfunctioning and damage of cellular organelles (Shabir et al., 2018).

In our study, Cd uptake increased in plant parts with increasing concentration in the soil while decreased when salinity was applied along with Cd. This is natural tendency in halophytes as is evidenced in previous investigations (Cheng et al., 2018; Lefèvre et al., 2009). These researchers noted that salinity application inhibited the Cd uptake by halophyte plant species: *Atriplex halimus* L. and *Carpobrotus rossii*. The activity of  $\text{Cd}^{2+}$  is decreased due to the arrangement of the  $\text{CdCl}_2$  network in the nutrient solution. This reduced activity of  $\text{Cd}^{2+}$  is one reason for limited Cd uptake by plants under NaCl stress (López-Chuken et al., 2012; Mariem et al., 2014). Few contradictory reports highlight an increase in the uptake of Cd due to the presence of the salinity (Rehman et al., 2019; Shabir et al., 2018), while others reported that interaction of salinity and Cd was concentration-dependent (Chai et al., 2013; Zhang et al., 2014). Still, the literature on the interaction of salinity and Cd under hydroponics is pretty variable and highlights that in addition to  $\text{Cd}^{2+}$  activity in solution, other

**Fig. 3** Effect of salinity (mM) and Cd ( $\mu\text{M}$ ) on activities of enzymes; SOD (a), CAT (b), APX (c), and POD (d) of quinoa plants exposed to different concentrations of salinity and Cd under hydroponics. The data are means of four replicates  $\pm$  SE. The values with different letter(s) are significantly different from each other at 5% significance level



mechanisms are also involved in the uptake of Cd under saline conditions (Cheng et al., 2018; Mariem et al., 2014; Wali et al., 2015). Another possible reason for the limited uptake of Cd might be the decreased  $\text{Cd}^{2+}$  activity on the plasma membrane in the presence of NaCl. For example, an increase in the concentration of cations such as  $\text{Na}^+$  resulted in reduction of negative potential at the plasma membrane, leading to reduced activity of metal ions, including  $\text{Cd}^{2+}$  (Wang et al., 2019). In comparison with bulk solution, the movement of Cd on the outer surface of the plasma membrane was more important for metal uptake by plants (Cheng et al., 2018). However, competition for the exchange of ions through different channels cannot be ruled out for limited uptake of Cd as noted by Mei et al. (2014) in *Amaranthus mangostanus* plants with reduced uptake of Cd in the presence of NaCl due to competition between  $\text{Cd}^{2+}$  and  $\text{Na}^+$  for  $\text{Ca}^{2+}$  channels. In our study, quinoa roots accumulated more Cd than shoots, which can be attributed to root-mediated production of the phytochelatins (PCs) and, ultimately, the formation of Cd-PCs networks. These Cd-PCs complexes are sequestered within root vacuoles, limiting the transfer of Cd from root to shoot (Cheng et al., 2018; Rehman et al., 2019; Zhang et al., 2020).

The bioconcentration factor (BCF) for Cd was  $> 1$  at all Cd concentrations, but the addition of NaCl decreased the BCF. Higher BCF values indicate that experimental plants had the potential to accumulate Cd. Contrary to this, in a soil culture experiment, Amjad et al. (2021) found  $\text{BCF} < 1$ , possibly because of less availability and uptake of Cd from the soil than hydroponics. The translocation of Cd from root to shoot (TF) was  $< 1$  for all the treatments, either alone or in combination with salinity. Restricted translocation of Cd and some other heavy metals due to the presence of salinity has already been observed in quinoa (Amjad et al., 2021; Iftikhar et al., 2021; Parvez et al., 2020). Lower TF values in quinoa indicate its phytostabilization potential for Cd under saline and non-saline conditions.

The overproduction of ROS ( $\text{H}_2\text{O}_2$  and TBARS) was initiated at lower levels of salinity and Cd, which increased at elevated levels of Cd (100 and 200  $\mu\text{M}$ ). The increased oxidative stress was demonstrated in decreased MSI due to lipid peroxidation of cell membranes. In line with our results, overproduction of  $\text{H}_2\text{O}_2$  and TBARS and resultant membrane damage

under salinity and Cd have been observed in different genotypes of quinoa (Amjad et al., 2021; Iftikhar et al., 2021; Parvez et al., 2020). Interestingly, at lower levels of salinity and Cd, oxidative stress and membrane damage were not aggravated. This might be due to the positive effect of salinity, which restricted the uptake of toxic Cd ions by plants. However, the joint application of higher doses of salinity and Cd caused more oxidative damage than their sole applications, perhaps due to excessive uptake of Na ions by plants at improved salinity levels in the growth medium (Iftikhar et al., 2021). Hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) is considered the most damaging among various ROS because of its conversion into noxious hydroxyl anions (Anwar et al., 2021). Therefore, proper mitigation of  $\text{H}_2\text{O}_2$  should be accomplished to prevent cellular damage. The detoxification of ROS is achieved through the activation of the antioxidant enzyme system within the cells (Abbas et al., 2021; Shahid et al., 2017a). Superoxide dismutase (SOD) serves as the primary defense system against the damage incurred by ROS because of its involvement in the detoxification of the superoxide radicals (Iftikhar et al., 2021; Natasha et al., 2020). These radicals are converted to  $\text{H}_2\text{O}_2$  and oxygen in the presence of SOD (Rehman et al., 2019; Shahid et al., 2017b). The activity of the SOD enzyme was considerably increased due to Cd stress which may lead to the overproduction of  $\text{H}_2\text{O}_2$  radicals and improved interaction of SOD and Cd (Natasha et al., 2021; Shahid, 2021). The detoxification of  $\text{H}_2\text{O}_2$  is accomplished through its conversion into water and oxygen in the presence of CAT, APX, and POD (Iftikhar et al., 2021). The enhanced enzymatic activities in quinoa under salinity and Cd stress indicate their role in mitigating the effects of  $\text{H}_2\text{O}_2$  in quinoa (Amjad et al., 2021; Rehman et al., 2019).

## Conclusions

The physiological attributes and growth of quinoa plants were reduced both under sole treatment of Cd or its joint application with salinity (300 mM NaCl). A moderate level of salinity (150 mM NaCl) relieved the quinoa plants from Cd-induced toxicity by inhibiting the aggregation of Cd and activation of the antioxidant enzyme system of plants. Increased tolerance and less uptake of Cd due to moderate salinity level showed

that the quinoa genotype named “Puno” was suitable for the phytostabilization and could be successfully cultivated in Cd-contaminated saline and non-saline soils.

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**Availability of data and material** Data will be available as demanded.

**Declarations**

**Conflict of interest** The authors declare that they have no conflict of interest.

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