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Full Length Article

Hyperaccumulation Response of Quinoa to Soil-Applied Cadmium: A Better Choice as Cadmium Phytoremediating Plant

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Abstract

Contamination of metals within the soil is the biggest issue in the wake of industrial development now-a-days. The easiest, effective and cost-effective way to cope with this problem is phytoremediation. Metallophytes such as *Chenopodium quinoa*, has a great potential to clean the metal pollutants from contaminated soils. This work assessed the potential of Quinoa plant growing on cadmium (Cd) contaminated (0, 100, 200, 300, 400 and 500 mM) soils using four quinoa lines (A1, A2, UAF-Q7, A9). Result showed an increase in morphological and physiological parameters i.e., chlorophyll and carotenoids contents at low levels of Cd and reverse was true for higher Cd levels. Phenolics contents were increased at high Cd levels. Cd accumulation in different parts of quinoa plant increased with increase in the levels of applied Cd. The yield attributes showed an increase up to 200 mM and further increase in Cd levels showed inhibitory effects. Quinoa lines UAF-Q7 and A2 performed better in all above attributes than A1 and A9. The results showed that *C. quinoa* could be a new candidate as Cd accumulator

Keyword: Phytoremediation; Hyperaccumulators; Halophyte; Quinoa; Cadmium; Yield growth

Introduction

Exorbitant amount of industrial waste discharge is increasing heavy metal toxicity in less developed areas of world. This problem would be a threat to sustainability of agriculture (Sabir *et al*. 2011). Above a certain concentration, heavy metals cause the reduction of yield by deteriorating plant growth (Jalloh *et al*. 2009). Human and natural activities deposit heavy metal in environment, and these metals are major cause of pollution (Gaur and Adholeya 2004). According to Appenroth (2010) the use of pesticides, smelting, mining, industries, rock erosion and many other anthropogenic activities are major sources of metal contamination. The issue of soil quality must be taken seriously as it is main growing medium for plants.

Due to its solubility and toxicity, Cadmium (Cd) is considered as major soil pollutant (Jain *et al*. 2007). Cd is easily assimilated by plants and in soil it can reach at high levels (Milone *et al*. 2003). Industrial waste, mining, pesticides and phosphate fertilizers are important sources of Cd (Jain *et al*. 2007). Other sources like combustion of fossil fuels and use of contaminated water change the productivity and quality of soil, which lead to reduce the yield of crop (Dourado *et al*. 2013). Biosynthesis of chlorophyll and membrane functions are affected due to Cd toxicity (Jain *et al*. 2007). As Cd is non-essential element, so it is very toxic even at lower concentrations (Vitoria *et al*. 2001; Milone *et al*. 2003). As the Cd salts is water soluble, Cd can be easily absorbed by plant roots and transferred to aerial parts (Daud *et al*. 2009). The consumption of these parts is a great risk for human and animals health (Kubo *et al*. 2016), since it is very toxic even at low doses (Li *et al*. 2014).

The management of these issues has become very important to minimize the effect arising due to Cd pollution on plant, soil and environment. However, it is very challenging because the techniques for cleaning soil are very costly and complex (Barcelo and Poschenrieder 2003). In this regard, many methods are used for cleaning soil to reduce the pollutants. These methods are electrokinetic, excavation, soil washing and incineration but all these techniques are very costly and damage the soil quality and fertility (Wuana *et al*. 2010). Phytoremediation is a method in which plants extract contaminants from soil by virtue of their natural abilities (Greipsson 2011). This technique not only removes metals from soil but also cleans environment from other pollutant e.g., PAHs, PCBs and pesticides. Phytoremediation is cost effective and ecofriendly technique (Kalve *et al*. 2011; Sarma 2011).

Members of Chenopodiaceae family have the ability to minimize metal contents in soil by accumulating them in

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leaf and other body parts (Zulfiqar *et al.* 2012; Haseeb *et al.* 2018). Bhargava *et al*. (2008) reported that quinoa (*Chenopodium quinoa* Willd.) is highly efficient among these species for Cd, nickel and chromium hyperaccumulation. The grains of quinoa have high nutritional value (Jacobsen *et al.* 2003). It can tolerate 40–80% humidity, can bear diverse climatic conditions (Valencia-Chamorro 2003). However, little is known about the Cd- phytoextractability of quinoa. The objective of the study was to find phytoextraction ability, morphological and physiological mechanism of *C. quinoa* under Cd toxicity.

Materials and Methods

Experimental location and source of seed

Experiment was carried out during Nov–Apr, of the years 2017–2018 in Old Botanical Garden, University of Agriculture, Faisalabad (32.41° N, 73.07° E), to explore the remediation ability of quinoa in different Cd contaminated soils. Four lines of quinoa (A1, A2, UAF-Q7, and A9) were used in this study. Seeds of quinoa were obtained from Department of Agronomy, UAF, Pakistan. Seed of four lines (A1, A2, UAF-Q7 and A9) were grown in pots in Completely Randomized Design with three replicates. Plants were treated at multiple leaves stage with six levels of cadmium chloride (0, 100, 200, 300 and 500 mM). Data were collected for growth and biochemical attributes after 15 days of cadmium treatment and yield variables data were documented at crop maturity.

Growth parameters

To collect data of morphological and growth variables two plants of each line (from each pot) were uprooted and shoots were separated from roots. Shoot and root length and their fresh weight were recorded. Plants were oven dried at 65^{o}C up to their constant weight and then dry weights were taken.

Physiological attributes

Arnon (1949) method was used for the estimation of chlorophyll contents. The contents of chlorophyll were extracted from 0.1 g fresh leaf at 4.0°C from each pot using 80% acetone. Then the extracted contents were placed overnight at 4^{o}C in darkness. The samples collected the next day and were then centrifuged at 10,000 rpm for 5 min. Supernatant absorbance was measured with a spectrophotometer (IRMECO U2020) at 645 nm, 663 nm.

Julkenen-Tiitto (1985) method was used for the determination of total phenolic content from leaf tissue. A 0.5 g leaf sample was extracted in 80% acetone (10 mL) and the material was then centrifuged at 10,000 rpm for 10 min. A 100 µL of supernatant was taken in a test tube and added 1 mL DW, 0.5 mL Folin Ciocaltue reagent and 2.5 mL of 20% $NaCO_3$ solution were mixed, vortexed briefly and kept at room temperature for 20 min. The absorbance of the colored complex was taken at 750 nm on a UV-Visible spectrophotometer. Acetone (80%) was used as blank.

Cadmium determination

Dry material (0.1 g each of leaf, root and seed) was taken into digestion flasks. Two mL sulfuric acid was mixed and incubated at room-temperature in each flask overnight. The samples were digested at 150°C for 40 min, then 2 mL of 35% H_2O_2 was poured in flasks, and reheated at 250°C; repeated the above steps, until the material become clear. This colorless solution was then diluted in volumetric flasks with distilled water up to 50 mL and then used for metal analysis. Atomic absorption spectrophotometer was used to assess contents of Cd.

Yield attributes

The length of the main panicles at crop maturity was measured as mentioned by Jacobsen and Stolen (1993). The seeds and panicles were dried on filter paper at 25–30°C. After 10 days, seeds were manually threshed, and weights were recorded using digital balance.

Statistical analysis

Each treatment was replicated three times under completely randomized design and two-way ANOVA of the data for each attribute was computed using the COSTAT Computer Program. Cadmium levels and quinoa lines were taken as factors and their interaction was determined. The treatment means showing significant differences were labeled with different alphabets.

Results

Growth parameters

Treatment of plants with increased Cd levels significant changed the shoot fresh biomass of quinoa. Interaction of Cadmium levels and all lines had significant ($P \leq 0.05$) effect. An increase in fresh shoot weight of quinoa lines was found up to 300 mM of cadmium while further increase in cadmium concentrations decreased shoot fresh biomass. Maximum weight was noted in UAF-Q7 at 300 mM and A9 showed minimum fresh weight at 500 mM Cd. All levels of Cd caused significant ($P<0.001$) variations in fresh biomass of root and combine effect of treatments and quinoa lines was non-significant. Root fresh biomass of all lines improved up to 200 mM and decreased at further increase in Cd concentrations. UAF-Q7 produced higher root fresh biomass up to 300 mM while at 400 and 500 mM A1 performed better (Table 1; Fig. 1). Shoot and root lengths were significantly affected by all levels of Cd in all lines quinoa but their interaction had non-significant value.

Table 1: Mean squares of analysis of variance of data for shoot and root fresh weights and lengths of four quinoa lines A1, A2, UAF-Q7 and A9 when treated with various levels of Cd

SOV	df	Shoot length	Root length	Shoot fresh weight	Root fresh weight
Treatment	5	345.59**	45.958**	5156.3**	0.5230**
Varieties	3	645.94**	37.118**	3563.3**	0.1940*
Treatment × Varieties	15	30.585ns	1.5636ns	123.260*	0.0450ns
Error	48	37.204	1.0273	63.294	0.0621

Significant at ** P<0.01; * P<0.05 and ns P>0.05

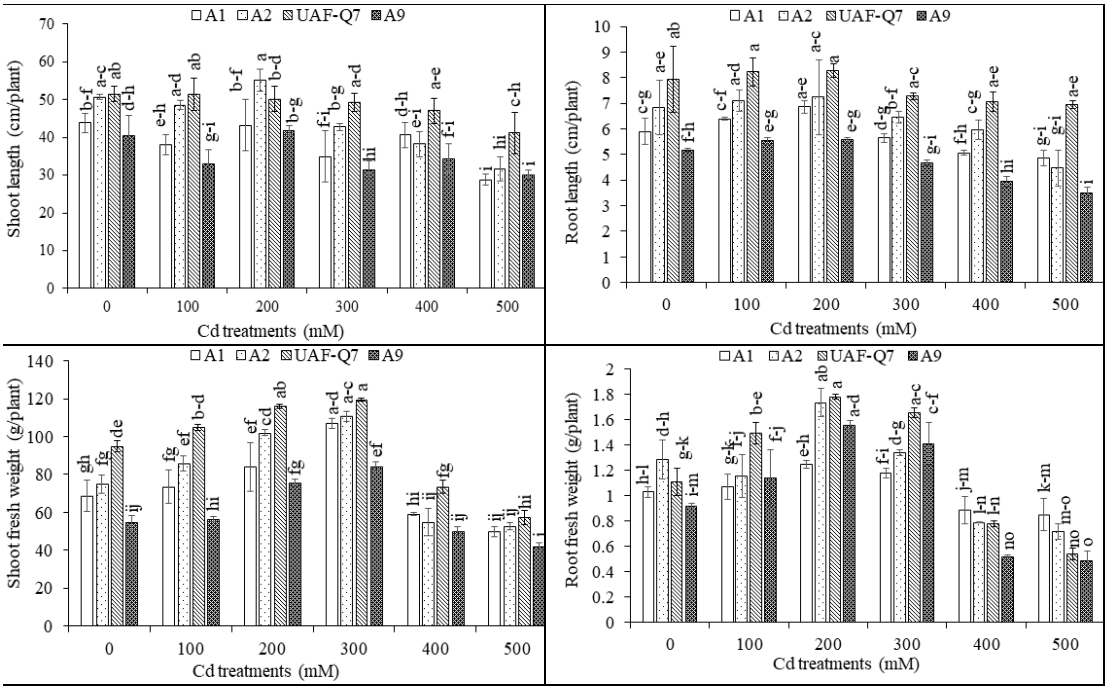

Fig. 1: Shoot and root fresh weights and root length of quinoa lines when grown under various levels of Cd stress

UAF-Q7 and A2 had more shoot length than A1 and A9 at all levels of Cd. 300 mM showed positive effect on this variable and further increase in Cd influenced negatively except A1 that showed more shoot length than A2 at 400 mM UAF-Q7 had maximum root length at all levels of Cd. It has been observed that root length steadily increased at 0 to 200 mM Cd then tended to decrease at further increased levels of Cd (Table 1; Fig. 1).

Physiological attributes

Chlorophyll *a*, *b*, *a/b* and total chlorophyll showed significant (P≤0.001) variations by Cd levels and quinoa lines while their interaction was non-significant for *b*, *a/b* and total chlorophyll and significant (P≤0.001) for chlorophyll *a*. UAF-Q7 and A2 had high amount of chlorophyll *a* and *b* than others. Maximum values in all lines of quinoa were found at 100 mM while at 500 mM all lines showed minimum values as compared to control. In current study all factors showed no variations on chlorophyll *a/b* ratio. Almost all lines showed same content of chlorophyll *a/b* ratio at all levels of Cd (Table 2; Fig. 2).

Cd and all lines of quinoa significantly affected phenolics and carotenoids. High levels of Cd decreased carotenoids and increased total phenolics in all lines of quinoa. The combined effect of quinoa lines and Cd concentrations was also significant for total phenolics. A2 and UAF-Q7 presented high amount of total phenolics as compared to A1 and A9 (Table 2; Fig. 2).

Cd accumulation

The Cd contents of roots and leaves showed significant Cd variations in all four lines and Cd treatment. Interaction of Cd and lines was also significant. Seed Cd content significantly influenced by Cd treatments. Cd contents in roots, leaves and seed directly linked with applied Cd levels. As conc. of Cd treatment increased, accumulation of Cd in different parts of plant increased. UAF-Q7 accumulated high content as compared to others. Leaves of quinoa accumulated highest amount of Cd while seeds accumulated lowest amount. In seed Cd content, A1 and A7 had more Cd than A2 and UAF-Q7 (Table 3; Fig. 3).

Table 2: Mean squares of analysis of variance of data for Chlorophyll *a*, *b*, *a/b*, total chlorophyll, carotenoids and phenolics of four quinoa lines A1, A2, UAF-Q7 and A9 when treated with various levels of Cd

SOV	df	Chl. *a*	Chl. *b*	Total Chl.	Chl. *a*/*b* ratio	Carotenoids	Phenolics
Treatment	5	0.6494**	0.0018**	2.2834**	2.9413**	0.1852**	0.0374**
Varieties	3	1.1841**	0.0022**	4.9208**	12.455**	0.3551**	0.0488**
Treatment × Varieties	15	0.0665**	0.0001ns	0.1646ns	0.2674ns	0.0099ns	4.3510**
Error	48	0.0286	9.5236	0.0414	0.2613	0.0058	1.324

Significant at ** P<0.01 and ns P>0.05

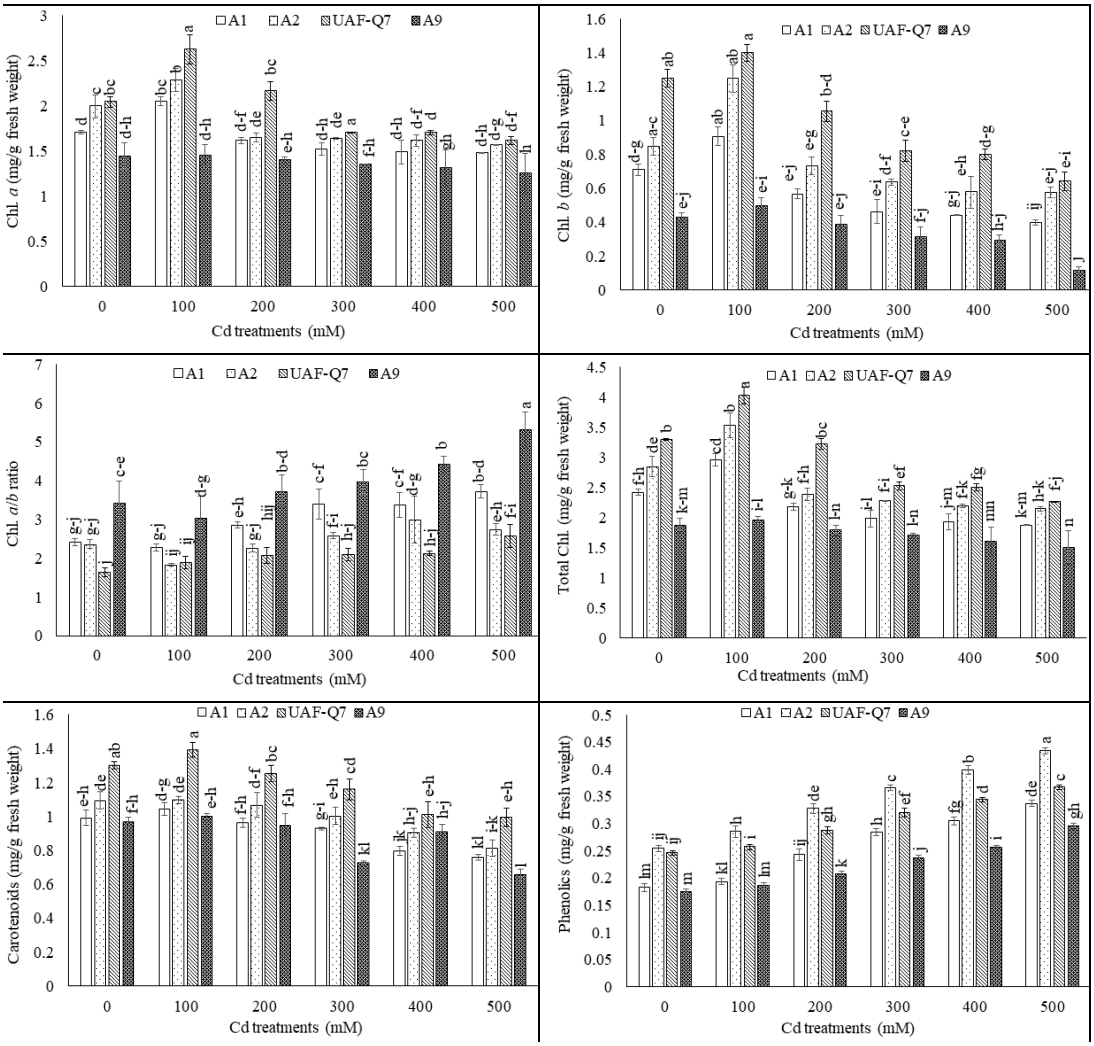

Fig. 2: Chlorophyll a, b, chlorophyll a/b ratio, total chlorophylls, carotenoids and phenolics contents of quinoa lines when grown under various levels of Cd stress

Yield attributes

Cd treatment significantly reduced the panicle length and grain weight while their cumulative effect was non-significant. Panicle length and seed weight increased up to 300 mM. Further level decreased the both parameters as compared to control (Table 3; Fig. 3).

Discussion

From this study it was observed that shoot fresh biomass is not affected by low Cd levels but slightly increased as opposed to power, so we can assume it is the optimum level for quinoa growth in the local environment. The weight of the shrubs under 100 mM increased (Tapia *et al.* 2011) and the low dose of Cd increased while the higher level of tomato biomass decreased (Rehman *et al.* 2011). Improvement in the growth of tomato seedling was noted under low concentration level of Cd. Treatment with Cd did not reduce shoot length (Rehman *et al.* 2011). The best criteria for a good hyperaccumulator plant, is that under metal contamination, its biomass above ground did not

Table 3: Mean squares of analysis of variance of data for root, leaf and grain cadmium contents, penicle length and 100-seed weight of four quinoa lines A1, A2, UAF-Q7 and A9 when treated with various levels of Cd

SOV	df	Root Cd^{2+} contents	Leaf Cd^{2+} contents	Seed Cd^{2+} contents	Panicle length	100-seed weight
Treatment	5	426.97**	872.189**	0.5900**	37.689**	2.3917**
Varities	3	39.986**	144.35**	0.0158*	9.3519**	1.2292**
Treatment × Varieties	15	3.9743**	10.485**	0.0022ns	0.2296ns	0.0181ns
Error	48	0.5109	0.0059	0.0056	1.2778	0.0825

Significant at ** P<0.01; * P<0.01 and ns P>0.05

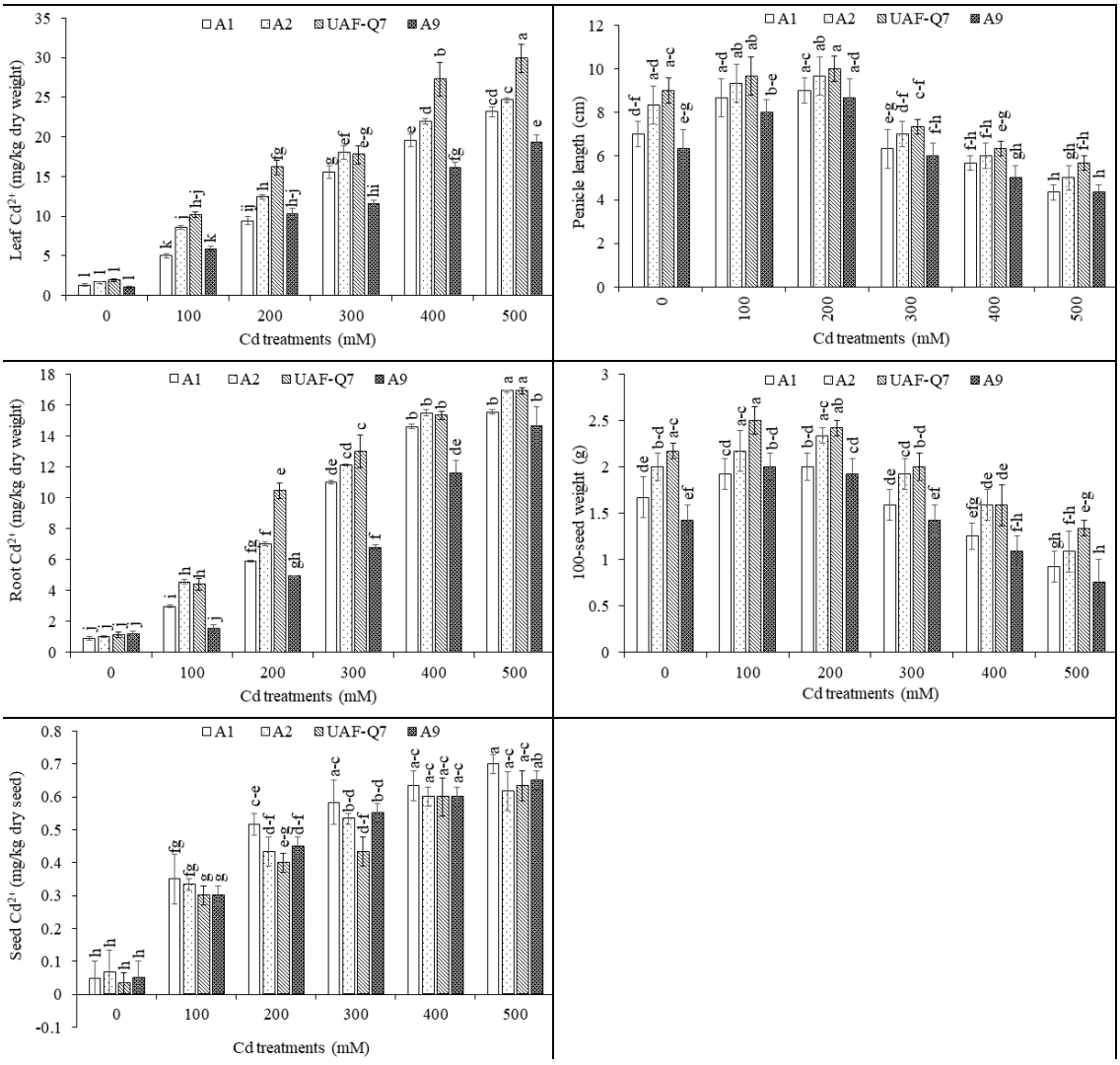

Fig. 3: Leaf, root and seed Cd^{2+} contents and panicle length and 100 seed weight of quinoa lines when grown under various levels of Cd stress

decrease significantly (Cui *et al.* 2013). No decrease in dry shoot weight and plant weight of *Solanum nigrum* L. (Cd hyperaccumulator) at 25 mg kg^{-1} Cd was observed (Sun *et al*. 2008). Slight increase in *Bidens pilosa* L. shoot biomass were reported at a concentration of 16 mg kg^{-1} of Cd (Sun *et al.* 2009). In moso bamboo, stem dry biomass did not reduce when grown at a Cd level of 5–120 mg kg^{-1} (Li *et al.* 2016). Exposure to Cd stress changed the biomass of Holm oak in controlled condition, but the high tolerance was observed in seedling with high amounts of Cd accumulated in roots and shoots (Dominguez *et al.* 2011).

In all quinoa lines, the chlorophyll and carotenoids contents increased at 100 mM and decreased with further doses throughout Cd levels. These data suggest that *C. quinoa* could reduce the damage to chlorophyll even at 100 mM. High quantity of carotenoids is reported to minimize oxidative damage from heavy metals (Unyayar *et al.* 2005). Improvement of the chlorophyll/carotenoids ratio may

play a defensive role as they are known as effective quencher for ROS, particularly for singlet oxygen (Caretto *et al.* 2002; Chaitanya *et al.* 2002; Tewari *et al*. 2002). However, carotenoids are also real ROS dousers, which are produced under stress conditions and played an important role as a light harvesting pigment. In tomatoes treated with Cd, the chlorophyll content increased considerably (Rehman *et al.* 2011).

In plants, total phenolics are of great importance because they are the major group of secondary plant metabolites. Phenolics are (non-enzymatic) antioxidants and have been involved in interaction with many abiotic and biotic factors (Reddy *et al.* 2004; Tomar and Agarwal 2013). In the present study, the increase in phenolic compounds observed with increasing the level of Cd. Phenolic contents synthesis increased with treatment of Cd in sunflower (Abd-Allah *et al.* 2015), tomato (Abd-Allah *et al.* 2016) and *Cassia italiaca* (Hashem *et al.* 2016). Cd application increased plant phenolic content in wheat (Tomar and Agarwal 2013), pharbitis (Wada *et al.* 2014) and *Vicia faba* (Dawood *et al.* 2014). Phenolics and their derivatives have an important role in promoting growth in plants under various abiotic and biotic stress conditions. The phenolics synthesis increases the antioxidants levels and contributes to cell wall formation and protects plants in stress outbursts (Ahanger *et al.* 2015; Hashem *et al.* 2016).

Cd deposition increased as its levels increased in roots, leaves and seeds. Seed had the lowest Cd content accumulated than the leaves and roots. Ali *et al.* (2015) reported the same findings that Cd content increased also in geminated seeds. Heavy metals absorption is associated with increasing metal concentration (Xiong and Wang 2005). Increasing concentrations of Cd improved leaf and root content of Cd (Muradoglu *et al.* 2015). Similar findings were observed in almond (Nada *et al.* 2007) and *Lepidium sativum* (Gill *et al.* 2012), where Cd content accumulation increased with higher levels of Cd application.

Conclusion

All quinoa lines showed tolerance to cadmium levels. They survived even the high levels of cadmium application but showed better growth up to 200 mM. UAF-Q7 and A2 showed better results as compared to A1 and A9. Hence, they are more efficient phytoremediator. The possible mechanisms involved are better growth, diverse morpho-anatomical features, cadmium sequestration with carotenoids and phenolics, metabolic adjustments and keep maximum nutrients in plant parts.

Author Contributions

AR, MA and SMAB planned the experiment. MS and SMAB interpreted the results. AR and MA made the write up and statistically analyzed the data. AR made the illustrations.

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