

EFFECTS OF CADMIUM STRESS ON MORPHOLOGICAL PARAMETERS IN DIFFERENT TOMATO (*SOLANUM LYCOPERSICUM* L.) GENOTYPES

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Abstract

One of the key retrain to crop floras specially vegetables is being the heavy metal burden left as industrial waste. Current study's emphasis on the identification of the greater parents for the development of hybrid varieties with higher yield and improved quality traits of tomatoes (*Solanum lycopersicum* L.) under cadmium stress condition. Thirteen genotypes of tomato were evaluated via completely randomized design using two levels of cadmium, i.e. 3 ppm, 6 ppm along with control. Important morphological growth traits of tomato plant such as length of root and shoot, weight of fresh and dry root, weight of fresh and dry shoot and metal contents in whole plant were assessed. The aim was to select best genotypes to be used in future breeding program on the basis of metal contents. All genotypes used in study exhibited alteration in studied traits at both levels of cadmium. Shoot length of genotype Roma and CLN-2123 A was least affected even at 6 ppm of cadmium. Root length of genotype Sitara TS-01 was least affected at 3 and 6 ppm levels while genotype CLN-2123 A stayed unaffected at 3 ppm while slightly reduced at 6 ppm. Fresh root weight was found higher in genotype Sitara TS-01 at both levels of cadmium. Genotype CLN-2123 A stored more metal contents in all body parts and showed least effect at dry root weight, dry shoot weight and root length while exhibited reduction in shoot length, fresh root and shoot weight. So genotype CLN-2123 A is suitable for bioremediation of soil while is not suitable for human consumption. Genotype Picdeneato had accumulated least metal contents at both levels, therefore it may be advised for human consumption although grown at both contaminated and sewage water irrigated soils.

Key words: Heavy metal, Cadmium, *Solanum lycopersicum*, Tomato.

Introduction

The prime threat to the health of ecosystems is heavy metal pollution. In contrast to organic chemicals, heavy metals cannot be eradicated by normal procedures just like their disintegration into compounds with low toxicity which produces distinctive challenges for their reclamation from soil, water and air (Atafar *et al.*, 2010; Nguyen *et al.*, 2021). Heavy metal pressure is a major constrain to crop plants particularly vegetables. In order to alleviate heavy metal oxidative stress, antioxidant mechanisms (e.g., tocopherols, glutathione, peroxidases, catalase, peroxide dismutase and ASC-GSH cycle) were up-regulated, mostly in the roots. (Alsherif *et al.*, 2022; Ammar *et al.*, 2008). Altered causes of metals like as urban and industrialized leftover (Rehman *et al.*, 2019, Sarwar *et al.*, 2020; Sayo *et al.*, 2020; Khan *et al.*, 2020), reprocessing of electronic waste (Luo *et al.*, 2011; Liu *et al.*, 2013; Yu *et al.*, 2019; Iqbal *et al.*, 2017; Han *et al.*, 2019), casting (Adimalla *et al.*, 2020; Trinh *et al.*, 2017), plant growth ions (Beygi and Jalali 2019) and chemical insect control components (Tsozue *et al.*, 2019; Qin *et al.*, 2021; Saleem, 2019; Salem *et al.*, 2020; Sultan *et al.*, 2019), mobile installations (Frigo *et al.*, 2020) but waste water/sewage water is a major

source of heavy metals in areas where sewage water is used for irrigation (Torghabeh *et al.*, 2019; Khan *et al.*, 2020) and metals composition differ from soil to soil depending on place. Waste water is extensively used for irrigation due to its vital economic and field significance. It is an additional source of water and it acts as a potential source to sustain soil fertility, augment farm production and reduce environmental pollution (Rashid *et al.*, 2005; Adesina & Awoyera 2019). As a source of fertilizer, urban waste water saves nearly US\$ 10.0 million in rising of crops (Ashraf *et al.*, 2018). Around periphery of big cities, 60% of the total vegetable production depends on waste water farming (Khan *et al.*, 2019).

Due to toxic biological effects of heavy metals, different strategies could be used to overcome the problems caused by heavy metals. Modern techniques for removal of heavy metals are very difficult and costly (Gong *et al.*, 2018; Qayyum *et al.*, 2020), so the effluents are mostly used unprocessed. Conventional technologies for subtraction of heavy metals from waste water are uneconomical (Yao *et al.*, 2011; Rehana *et al.*, 2019) and too expensive for the developing country like Pakistan. The use of substances also bases to reduced biological action and fecundity (Kaurin *et al.*, 2018). The only method is the

use of such plants which gather heavy metals in their tissues. But the concern of crop plants is important as if there is translocation of metal to edible portion, it will cause health problem after bio-magnification. Cadmium accumulation behavior varies on the species level, cultivar level and even at individual plants level. Cadmium is brought up by plants as Cd^{2+} and its amount in normal plants ranged from 0.1-2.4 milligram per kilogram and at higher amounts it severely lowered plant growth and dry biomass production. The low amount of Cd in the organism tissues at which it has damaging effects is five milligram per kilogram (Raza *et al.*, 2022; Li *et al.*, 2020). Soil Cd negatively affected sassafras growth and decreased the net growth of plant height and the biomass of leaf, branch and root. Significant decrease were observed in root biomass by 18.18% (Cd5), 27.35% (Cd20), 27.57% (Cd50) and 28.95% (Cd100) (Zhao *et al.*, 2012). Two of the grafting combinations of tomato from Cd stressed plants yielded seeds that generated seedlings with enhanced dry mass when they were sown in Cd-free media (~41%), suggesting a Cd-induced trans-generational enhancement of biomass production (Nogueria *et al.*, 2021).

Selected cultivar of tomato (*Lycopersicon esculentum* cv. Navodaya) were reported to be sensitive to higher levels of cadmium. The flowering was found to be more sensitive as compared to the rate of fruit setting whereas vegetative growth i.e. shoot length was affected only by high concentration of cadmium (Rehman *et al.*, 2011). Presently there are numerous radicals which interact with Cd uptake by plants. Therefore, tomato (*Solanum lycopersicum* L.) may be used as a model plant for genetic studies applicable for other species with flesh berry fruits (Li *et al.*, 2017; Han *et al.*, 2019). Li *et al.*, 2020 reported increased peroxidation of lipids, catalase activity, glutathione reductase (GR) activity and reduced glutathione peroxidase (GPX) activity in tomato plant under cadmium stress. The catalase activity was improved in the fruits when plants were salted with cadmium chloride. Therefore, need of research in more detail for the production of compounds which may be changed in the presence of cadmium in the plant (Chu *et al.*, 2020) must be carried out. Local adaptation and microevolution can be exemplified by natural selection of heavy metal tolerant ecotypes. Occurrence of ecotypes on mines having metals, support that evolution must be present in those ecotypes for metal tolerance.

Determination of genetic variation found in tomato genotype for bioremediation could be achieved by testing them at safe and toxic levels so that future planning could be done to germinate and plant these genotypes for safe human consumption. Therefore, the objective of current study was to investigate the character of inheritance of cadmium accumulation in tomato plants and its possible impact on agronomic traits.

Materials and Methods

Plant material: The research study was conducted at department of Plant Breeding and Genetics, University of

Agriculture, Faisalabad, Pakistan. Nursery of 13 tomato genotypes was raised up in organic matter-filled trays of size 15 × 15 cm. Seeds of these tomato genotypes was raised in growth chamber (16 h light/8 h dark) having mercury lamps with light intensity of 150 mmol/m² per sec., day/night temperature of 25/20°C and 65(95)% water contents. Forty-day old nursery of thirteen genotypes was shifted in plastic bags each of size 30 × 10 cm; filled with one kg soil having pH 7.6, organic matter 1.3%, sand:silt:clay (47:22:31), electric conductivity (2.026 dS m⁻¹), total soluble solids (22.6 mmol L⁻¹), Cl⁻ (8.25 Me L⁻¹), Pb (0.269 ppm) and Cd (0.046 ppm). Trial was conducted in triplicate.

Experimental design: Complete Randomized Design with two factors (factorial) with three treatments. Six plants were used in each replication for each genotype. Thus, the whole experiment was arranged in completely randomized design in glass house. Water holding capacity of soil was determined as 250 ml/Kg of soil. After one week of transplanting, salt of cadmium chloride (CdCl_2) was applied in solution form (250 ml) only once at concentration of 3 ppm and 6 ppm along with control (no salt). Normal agronomic (watering, hoeing, fertilization (i.e. NPK20:20:20) and plant protection measures (pesticides and fungicides) were adopted during the whole experiment.

Measurement of morphological traits: Seventy days after transplanting, Measurement of root length and shoot length was done with the help of scale for 6 plants in each genotype in each replication. Weight of fresh root and shoot was calculated with the help of electric balance after digging plants from soil. By oven drying roots and shoots, dry root and shoot weight was calculated.

Heavy metal analysis: With some changes, fruits, leaves, roots and shoots were collected in paper bags independently and moved to oven for drying. Plant samples were exposed to heavy metal analysis according to Osmolovskaya *et al.*, (2018). After drying, samples were ground to fine powder and half gram of each sample was used for digestion purpose using tri-acid method i.e. HNO_3 , HClO_4 and H_2SO_4 in 1:1:1 ratio to form digestion mixture. Digestion was done on hot plate keeping temperature 100°C for first hour, 150°C for 2nd hour, 200°C for 3rd hour and 250°C for 4th hour using 15 ml of digestion mixture. Digested samples were filtered with Whatmann's filter paper No. 42 and by adding distilled water, volume was made accordingly. The samples were kept in air free plastic bottles and exposed to heavy metal analysis using atomic absorption spectrophotometer (Model Thermo Electron S-Series), Thermo Fisher Scientific Inc. USA.

Statistical analysis

The individual plant data regarding all the traits were analyzed using analysis of variance technique (Ferreira 2019) to determine the significance of genotypic differences regarding heavy metals stress.

Results and Discussion

It has been observed that heavy metals can stimulate plants to produce more reactive oxygen species (ROS), produced ROS react with lipids, proteins, nucleic acids and other substances causing lipid peroxidation, membrane damage and enzyme inactivation, thereby affecting cell performance and viability. Cadmium (Cd) is highly mobile in plant tissues and affects plant physiological growth. Cd toxicity is known to cause a deleterious effect on plants by disturbing the overall physiological mechanisms of plants. Once the ability of the plant to clear Cd itself is exceeded, it will even cause the death of plant. Therefore it is crucial to assess the bioremediation and stress defense strategies of a tolerant plant species. The shoot length was greatly affected by increased cadmium application and its effect was additionally prominent in genotype 17882 (Fig. 1), whereas genotype Roma and CLN-2123 A were least affected by increased concentration even at 6 ppm. Higher dose (40 μg Cd) reduced the shoot length of tomato (*Lycopersicon esculentum* cv. Navodaya.) plant significantly (Rehman *et al.*, 2011). On an average of the 2 tomato cultivars, exposure to 1 and 10 $\mu\text{mol/L}$ Cd for 33 days reduced plant height by 18.9% and 46.4% and SPAD value by 11.2% and 31.6%, compared with control, respectively (Dong *et al.*, 2005). Torghabeh *et al.*, (2019) mentioned that plant taxonomy also influences shoot heavy metal concentration and salt tolerant taxa do differ from salt sensitive ones in their heavy metal accumulation pattern, the former group being more suitable for decreased heavy metal polluted land. It is reinforced that salt tolerant plants characteristically move and store minerals in vacuoles in the leaf tissue, so apparently translocate ions from roots to shoots more effectively than salt sensitive plants (Sayo *et al.*, 2020).

Increased root length was found at control condition in genotype 6231 (Fig. 2). Genotype Marmande was most affected at 6 ppm level while its length was increased with reference to control at 3 ppm. Genotype Sitara TS-01 was least affected at 3 and 6 ppm levels while genotype CLN-2123A remained unaffected at 3 ppm while slightly reduced at 6 ppm (Hussain *et al.*, 2015).

Rehman *et al.*, 2011 reported that the root length of tomato plant treated with 40 μg of Cd also reduced significantly. The root length of tomato cultivar exposed to 1 and 10 $\mu\text{M/L}$ Cd for 33 days was reduced by 41.1% and 25.8% and root volume by 45.2% and 63.7%, respectively (Dong *et al.*, 2005). It has been reported by Alamri *et al.*, (2020) that the higher dose of Cd (25 μM) caused greater decline in root growth, endogenous H₂S and phytochelatin, activities of DES, glutathione biosynthesis and AsA-GSH cycle enzymes, disturbed redox status of ascorbate and glutathione which collectively led to higher oxidative stress in tomato roots. The results showed a negative effect of cadmium, at different concentrations (12 and 25 μM) on root, shoot dry weights and root morphology (Naciri *et al.*, 2021). The results indicated that the shoot and root growth on all Cd concentrations (5-100 μM) was reduced (Heybet *et al.*,

2020). Moreover, fertilizers affect Cd ions and complexation, which cause suffering of the translocation of Cd to plant roots and maybe also its absorption by the roots. In addition, rhizosphere structure, root development and overall crop progression are likely to be affected by the application of nourishments (Ashraf *et al.*, 2018). Cadmium is attached to cation exchange sites of maize root tips having mucilage excretions (Qin *et al.*, 2021) and to places in root cell walls (Li *et al.*, 2017). Binding of Cd to the sites would be probable to reduce Cd carriage in to the cell. Another favorable motivation is that the augmented concentration of Ca²⁺ ions decreased the binding of Cd²⁺ at spots in the root cell walls.

Fresh shoot weight was highest at control in genotype Roma and 17883 (Fig. 3). Genotype CLN-2123 A remained high for fresh shoot weight at 3 and 6 ppm with reference to control. Significant reduction in fresh shoot weight occurred at 6 ppm level in genotype 9086. Kumar *et al.*, (2015) found reduction in biomass in tomato plant by exposures to cadmium even at low concentration of 1 μM . The plant biomass decreased significantly on treatment with higher doses of Cd as reported by Rehman *et al.*, 2011. Though not necessary for plant growth, Cd is readily absorbed by roots and move to leaves. Due to the reduction of enzyme activity, photosynthesis, respiration, and nutrient uptake, high concentration of Cd caused growth inhibition of roots and shoots, leaf roll and chlorosis (Gratao *et al.*, 2008; Qayyum *et al.*, 2020).

Fresh root weight was found higher in genotype Sitara TS-01 at all levels of Cd. Non-significant reduction occurred in genotype 9086 at 3 and 6 ppm Cd (Fig. 4). Similar behavior was found in genotype Marmande. According to Rout *et al.*, (2000) the genotype responded in a different way related to seed germination, elongation of shoot and roots and their biomass construction. Accumulation of Cd in roots and shoots of some cultivars of Mung bean and rice were found to be higher than that in others. Cadmium buildup was high in shoot (stem + leaves) than roots of all the genotypes. It was clear that cadmium tolerant genotypes of Mung bean and rice indicated reduced shoot to root green biomass ratio in the presence of cadmium related to the control, i.e. the root biomass was higher than the above ground biomass. The researchers claimed that it will be due to larger movement of cadmium to roots because of non-existence of metal absorption by fixed or soluble chelators in roots, or to exchange with Ca, Mn and Zn moving through roots.

Dry shoot weight was found higher in genotype Sitara TS-01 in control while non-significant reduction in genotype Marmande was observed at all levels of Cd (Fig. 5). Genotype 17883 and BL-1176 remained unaffected at 3 ppm level. In genotype BL-1176 dry root weight was found higher than others. Non-significant reduction of root weight was found in all genotypes at 3 ppm while at 6 ppm slight reduction was observed (Fig. 6). Kumar *et al.*, 2015 found that at high concentrations of cadmium, an apparent increase in lipid peroxides is detected which shows that cadmium stress encouraged oxidative stress by barring lipid biosynthesis way.

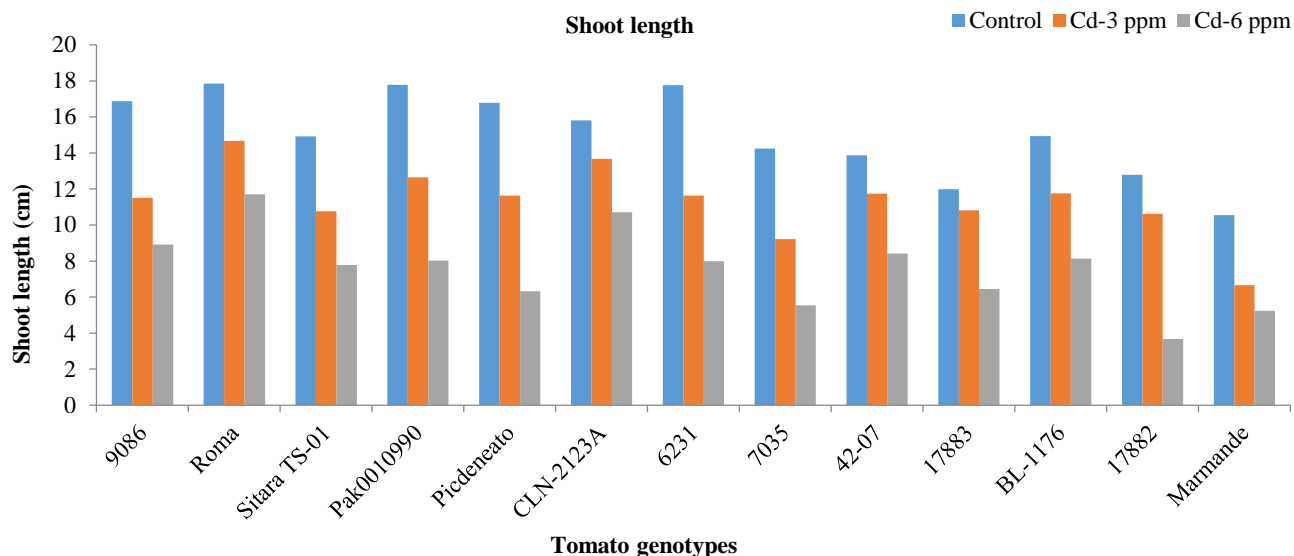


Fig. 1. Effect of Cadmium on shoot length of various types of Tomato genotypes.

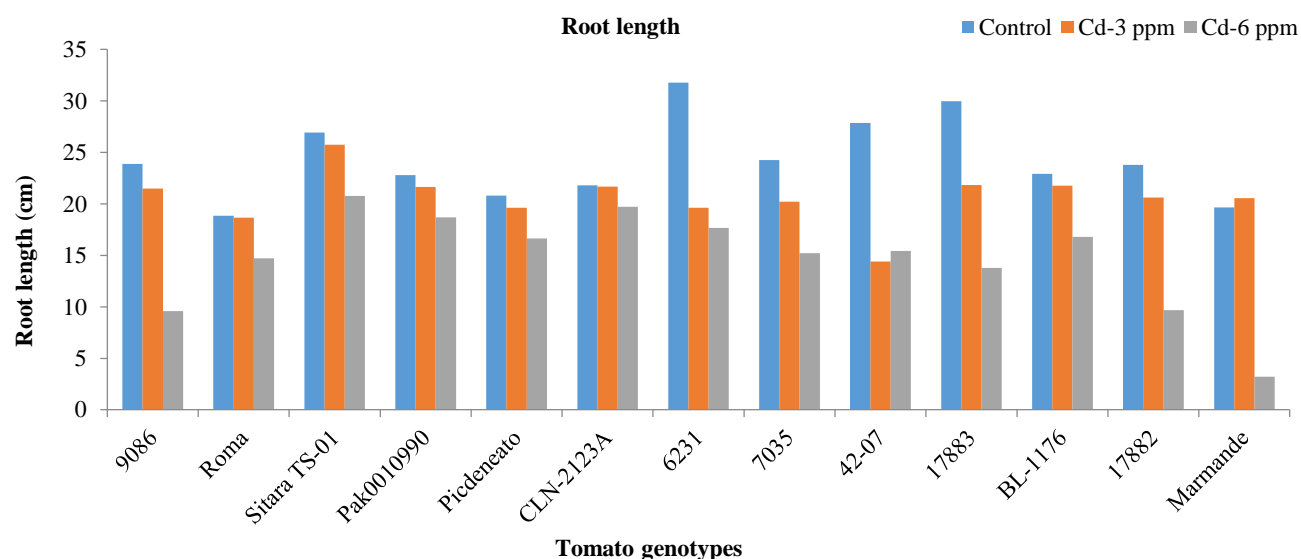


Fig. 2. Effect of Cadmium on root length of various types of Tomato genotypes.

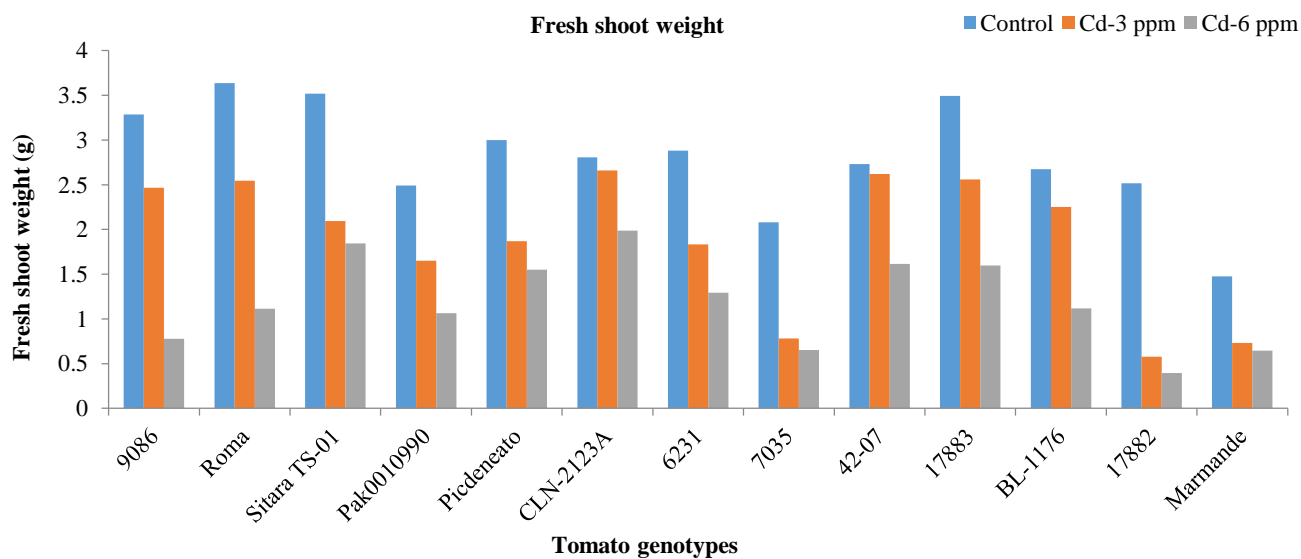


Fig. 3. Effect of Cadmium on fresh shoot weight of various types of Tomato genotypes.

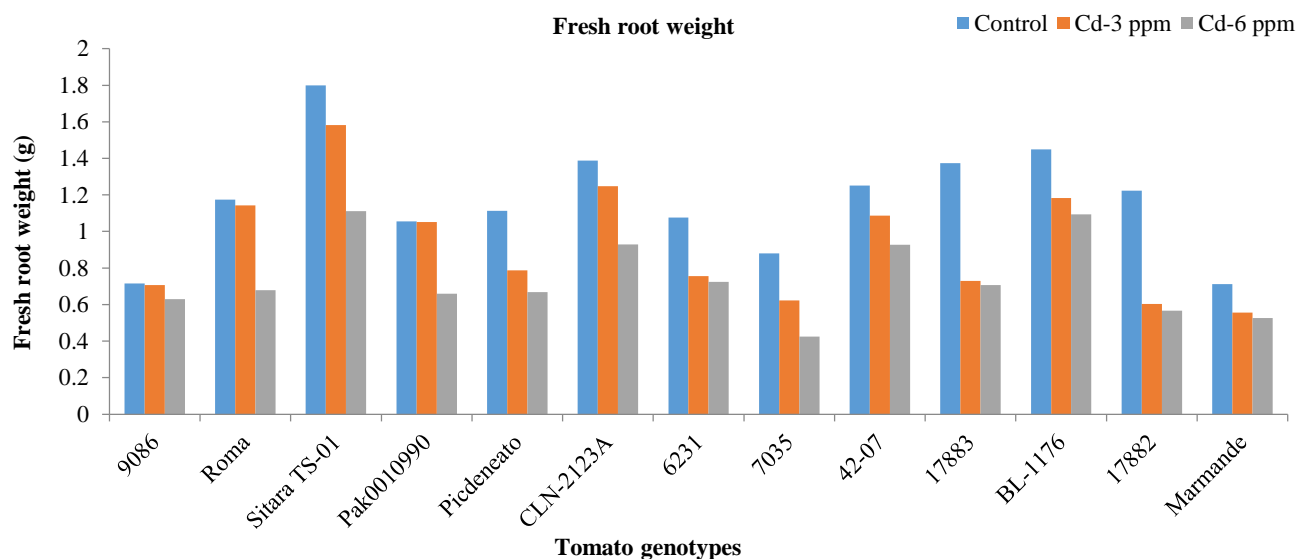


Fig. 4. Effect of Cadmium on fresh root weight of various types of Tomato genotypes.

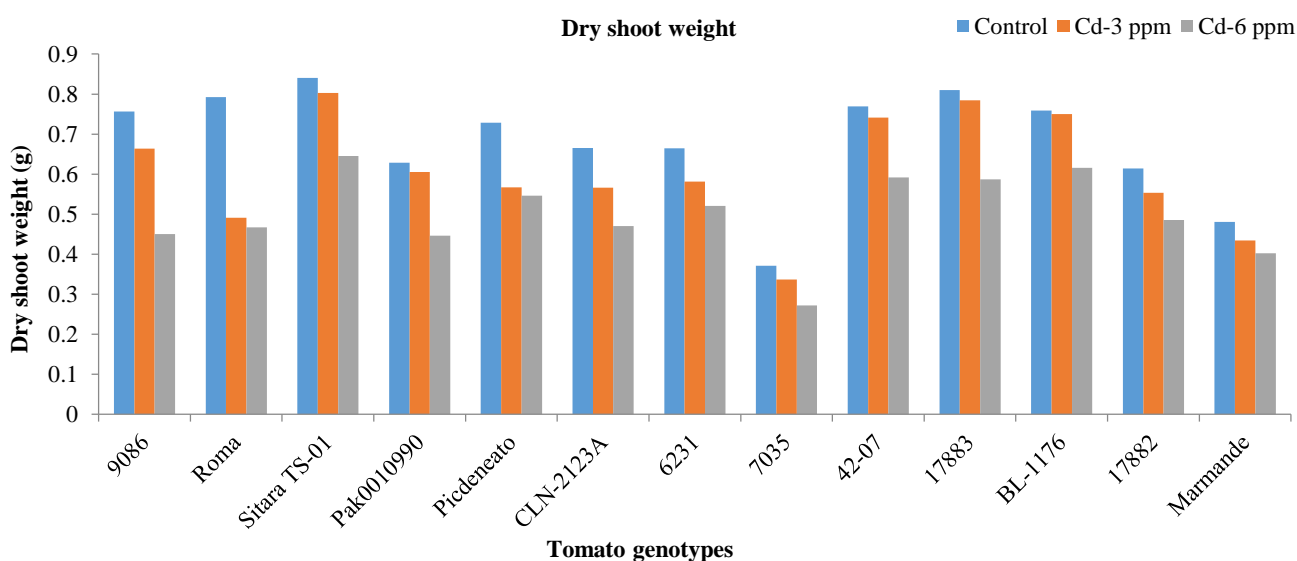


Fig. 5. Effect of Cadmium on dry shoot weight of various types of Tomato genotypes.

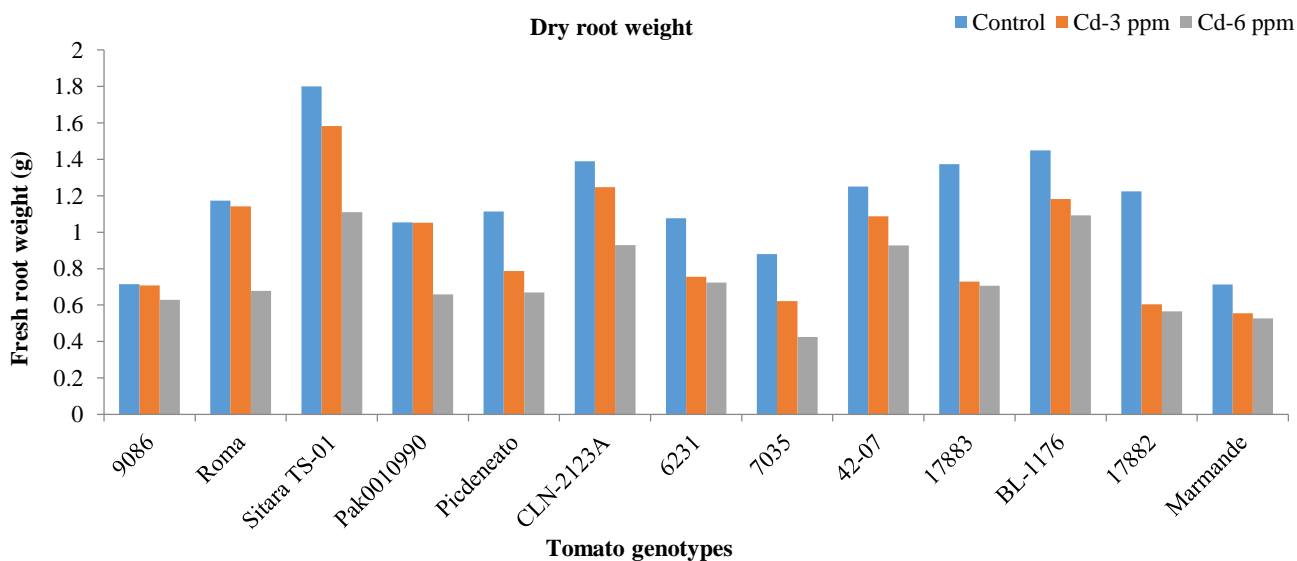


Fig. 6. Effect of Cadmium on dry root weight of various types of Tomato genotypes.

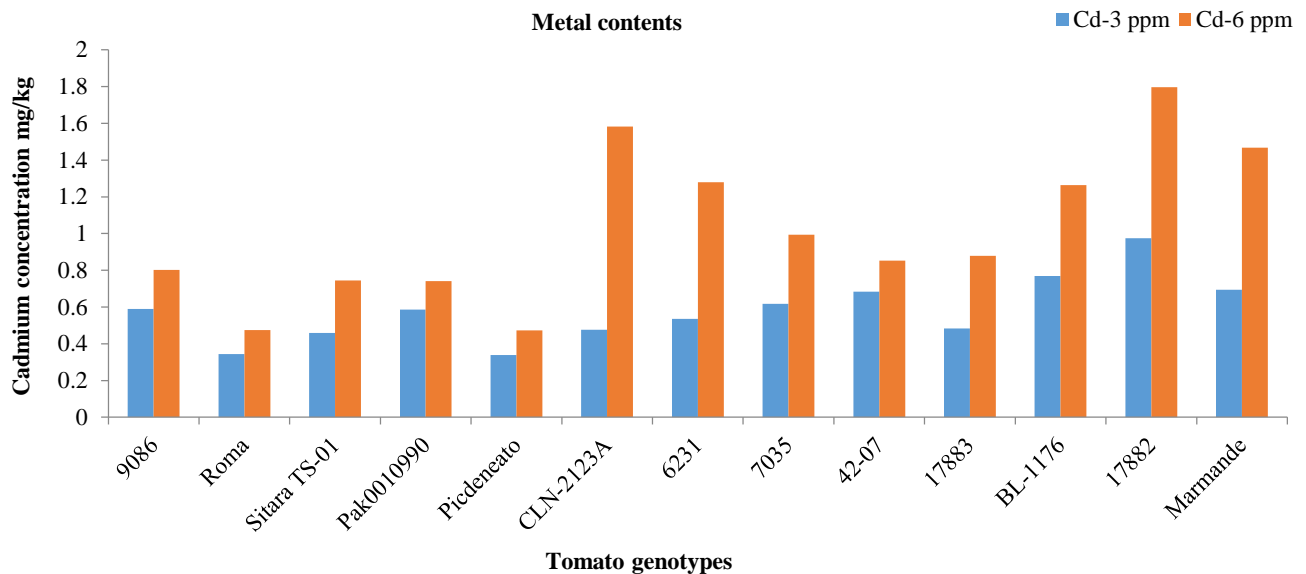


Fig. 7. Cadmium content (mg/kg) of various types of Tomato genotypes.

All genotype stored more metals contents with increased metal concentration in soil. Metal content were found higher at 3 ppm in genotype 17882 while higher at 6 ppm in genotype CLN-2123 A (Fig. 7). Kumar *et al.*, 2015 established that the maximum accumulation of cadmium in root even at low concentration of 1 μm . But can also be trans-located to the upper parts of the plant containing fruits on the other hand the absorptions in leaves are lower than those detected at last date of plant growth. After 75 days, the absorptions of cadmium in fruits were maximum of three organs, proposing that following the start of fruit enlargement cadmium is shifted specially to fruit, while all CdCl_2 actions brought amplified catalase activity in the fruits (Afzal *et al.*, 2016). Consequently, it is essential that research examined in more detail the biosynthesis of phytochelatin and even other antioxidant pathways and metabolites, which may also have been altered in response to cadmium in plants (Chu *et al.*, 2020). Increased glutathione reductase activity in the fruits indicating the fruit tissue was significantly affected by cadmium. This would perhaps be predictable because the growth of the fruit only happened at a short time before the plants were tested (Kurtyka *et al.*, 2008). So, they mentioned that rhizofiltration is the use of plant roots to eliminate contaminants, as metals from polluted water may have effects on developing the ecological cleanup technology. The metal absorption by roots and shoots of the tomato may be subjective to an exclusion mechanism initiated by high outer cadmium concentrations.

Conclusion

The findings of the current study revealed that genotype CLN-2123A accumulated significant metal contents in all parts of plant. The dry root weight, fresh root weight, fresh shoot weight, dry shoot weight and root length were least affected by Cadmium. So genotype CLN-2123 A is suitable for bioremediation of soil. Genotype Picdeneato exhibited least metal contents; hence it could be advised safe for human consumption.

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