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Genetic evaluation of root and seedling growth of tomato genotypes under cadmium stress

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Abstract

Solanum lycopersicum L. is the most important crop in Pakistan, and concerns are being raised regarding the impact of cadmium (Cd) on tomato plants. This study aimed to determine the genetic differences between twenty tomato genotypes under Cd stress by comparing their root and seedling growth to determine which genotype was the most tolerant to Cd stress. This field experiment was carried out during 2020-2021 in the screen house of the Department of Plant Breeding and Genetics, University of Agriculture Faisalabad, using a completely randomized design (CRD) with two replications. Genotypes were exposed to two treatments: treatment of plants with the specific element under consideration and a stress condition for that element; PT = control (0 ppm) and Cd stress (6 ppm). Various morphological traits were measured, including plant height, root/shoot length, and biomass. Statistical analysis was done by measuring internal consistency through principal component analysis and variance analysis. The results revealed significant differences among genotypes and identified Cd-tolerant tomato varieties suitable for breeding programs. The performance of genotypes was assessed based on key growth parameters, including root length, shoot length, biomass (both fresh and dry), and plant height. Notably, genotypes 17868, 17874, and Money Maker performed well under control conditions, exhibiting superior biomass production and root length. In contrast, Naqeeb and Cchaus showed poor performance, particularly in terms of biomass accumulation and shoot growth, indicating their reduced ability to thrive under optimal conditions. Under Cd stress, genotypes 19860, 17868, and 19865 exhibited favorable performance, primarily in terms of biomass accumulation and root growth, as it maintained relatively higher fresh root weight and shoot biomass compared to other genotypes (Naqeeb and 19899) under the same stress conditions. This study contributes valuable insights into identifying Cd-tolerant tomato genotypes, offering potential improvements for tomato cultivation in Cd-affected regions of Pakistan.

Keywords: Cd stress, Genotype screening, Heavy metal, PCA, Screening tomato

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Introduction

Due to several biotic and abiotic issues, agriculture has been severely affected (Zaman & Qureshi, 2018; Iqbal & Qureshi, 2021). A vast gap in production is present due to limited appropriate technology, improper time of using inputs, water availability, and lack of proper information. All these issues considerably decline the production level (Egea et al., 2022). Tomato plant, also known as *Solanum lycopersicum*, belongs to the Solanaceae (nightshade) family and contains 2n=24 chromosomes in its genome. The Solanaceae family contains about 100 genera (Shah et al., 2019; Rezk et al., 2021; Sanatombi, 2024). Tomato crop is a versatile crop of the world. It has high nutritional value and is a protective food (Sattar et al., 2024). The crop is widely produced throughout the world. Steroidal alkaloids, flavonoids, saponins, glycosides, carotenoids and derivatives of fatty acids are highly present in the tomato plant, which is why it is the reason for its fleshy cultivation (Waheed et al., 2020). Lycopene is one of the major components in tomato plants. It helps reduce the chances of heart disease and cancer and is an antioxidant (Przybylska & Tokarczyk, 2022). It is among the most cultivated vegetables in Pakistan, contributing significantly to domestic consumption and export. As of 2023, Pakistan is one of the top 20 tomato producers globally, with an annual production exceeding 6 million metric tons (FAO, 2023).

Heavy metal contamination in soil is one of agriculture's most significant environmental challenges. Metals such as cadmium, lead, arsenic, nickel, and chromium are significant pollutants that accumulate in the soil through industrial activities, agricultural practices, and pollution (Rashid et al., 2023). These toxic metals can severely affect plant growth by interfering with essential nutrient uptake, photosynthesis, and root development (Altaf et al., 2021). For example, cadmium can reduce root growth and lessen biomass accumulation,

while lead and arsenic can lead to oxidative stress that is directly detrimental to plant cells and tissues (Zhu et al., 2021). Globally, more than 30% of agricultural soils contain heavy metals, and cadmium is ranked at the top of the list (Alengebawy et al., 2021). This contamination negatively impacts yielded crop production and nutrient value, becoming perilous to food security and human health. Some trace metals such as copper, iron, and zinc are essential micronutrients for plants but can also cause bioaccumulation in the food chain when present in excessive amounts (Uddin et al., 2021). Soil pollution is mainly caused by heavy metals, particularly cadmium (Genchi et al., 2020). Other than metals, non-metals and phenolics are also in the soil. Heavy metals have become common in soil and pollute it due to increasing agricultural activities. Recently, it has become a significant concern in agriculture because of its effects on crop growth and other sectors (Asati et al., 2016). Thousand tons of fruits and vegetables are produced in Pakistan. Metal contamination affects significant crops in Pakistan such as tomato, rice, cotton, potato, eggplant cucumber, wheat, and carrots (Khan et al., 2022; (Saeed et al., 2023). These crops contain high amounts of protein, vitamins, iron, calcium and carbohydrates, but the profile of all these nutrients is being affected due to the accumulation and transport of metals (Iqbal et al., 2018).

Cadmium, the most prevalent heavy metal in soil in Pakistan, has been found to have adverse effects on the growth and production of major crops. Bio-accumulator use in industries has increased in recent years, resulting in an accumulation of cadmium in the soil (Zulfiqar et al., 2022). Plants readily take up metals such as cadmium, which can cause significant changes in tissues and metabolic pathways, even at low concentrations, leading to alterations in root morphology and limiting the transport of essential nutrients such as calcium, iron, copper, zinc, and manganese (Ma et al., 2015). Cadmium competes with these nutrients, reducing their transport to other plant parts. Like other toxic heavy metals such as arsenic, mercury, and chromium, cadmium (Cd) has no known physiological function in plants and is considered a toxic metal (Zhu et al., 2021). Cadmium is derived from various anthropogenic sources, including fossil fuel combustion, smelting of copper and nickel, and phosphate fertilizers. It can also be found in non-ferrous metal smelters and electronic waste recycling. Cadmium accumulation in roots may occur due to chelation with cellular ligands and subsequent sequestration in vacuoles, as well as the development of extracellular barriers that restrict its translocation through the root symplasm, particularly in acid soils where its solubility is increased by root exudates (Lux et al., 2011; Pasricha et al., 2021).

The increasing accumulation of heavy metals in soil is a worldwide concern in agriculture and related sectors, as cadmium is highly toxic and poses potential risks to human health and ecosystems (Fernandez-Luqueno et al., 2013). Approximately 70% of cadmium availability is through plant-based food, leading to its transfer to humans, and long-term exposure to cadmium in food has been linked to various health issues (Yamaguchi et al., 2011; Chang et al., 2014). The toxicity level of cadmium in plants is influenced by bioavailability, temperature, pH, redox potential, and other soil conditions (Irfan et al., 2014). The increasing soil contamination with heavy metals, particularly cadmium, has a detrimental impact on crop growth, including tomatoes. While previous studies have focused on the general effects of cadmium on plants, there is limited research on the specific response of different tomato genotypes under cadmium stress, particularly in regions like Pakistan. The objectives of the present study were to screen tomato genotypes for their response to cadmium stress at two levels (control and treated) and to characterize their morphological traits under cadmium stress conditions.

Materials and Methods

Research material

The screen house of the Department of Plant Breeding and Genetics (PBG), University of Agriculture, Faisalabad (UAF), was selected to experiment during the winter of 2020-2021. Twenty tomato (Table 1) genotypes were screened under normal and 6 ppm cadmium levels. Seed materials for the genotype were obtained from the the Vegetable Research Institute (AARI), the seed bank of the Department of PBG, UAF, and Nuclear Institute for Agriculture and Biology (NIAB). The selected genotypes did not undergo prior selection based on their response to cadmium stress but were chosen randomly to cover a diverse range of tomato varieties. This selection aimed to capture genetic variation in response to cadmium stress, allowing for the identification of genotypes with varying tolerance levels.

The seeds were sown using a two-factor factorial under a completely randomized design (CRD) with two replicates. Two cadmium levels (normal and treated) were given per replicate, viz. T0 (normal) and T1 (6 ppm Cd). In contrast, 6 ppm concentration was chosen based on prior studies showing its significant impact on tomato plant growth and simulating environmental contamination levels. First, twenty tomato genotypes with two replications were sown in the soil during the cropping season of 2020. For 3 weeks, the field was irrigated with controlled water, although the exact percentage of controlled water used (e.g., 24%). At the same time, polythene cups (height: 11 cm, width: 45 cm) were filled with growth media (approximately 800 g of sand/cups) and placed in the glass house of the Department of PBG, UAF. After 3 weeks of emergence, healthy and uniform-sized plants were transplanted from soil to polythene cups. By using the lottery method, each plant was transplanted into cups.

Table 1 List of 20 genotypes of tomato used for an experiment

Sr. No.	Genotype name	Sr. No.	Genotype name
1	17868	11	19860
2	CLN-3552D	12	17874
3	Kanatoo	13	19865
4	17266	14	19895
5	17272	15	19897
6	18278	16	19899
7	Naqeeb	17	19900
8	19908	18	Peto 86
9	19903	19	Money Maker
10	19857	20	Cchaus

Treatments

After one week of transplantation, plants were given two cadmium treatments, control (0ppm), and stress (6ppm) in polythene cups. Cadmium was applied in the form of Cd. It was necessary to apply cadmium treatment after 1 week of transplantation to avoid transplanting shock. Hoagland's solution (Table 2) was used to get accurate and fair results for both levels of treatment. Through Hoagland's solution, cadmium stress and nutrients were given to plants. After irrigating plants on alternate days with simple Hoagland's solution for at least 1 week, Hoagland's solution containing 6ppm of cadmium was applied to plants in polythene cups. Hoagland's solution was prepared following measurements given below:

Table 2 Components for preparation of stock solution and half-strength Hoagland solution

Components	Stock solution (1L)	mL Stock/ 1 L of Hoagland solution
Macronutrients		
1 M KN_3	101.1 g	2.5
1 M $CA(NO_3)_2.4H_2O$	236.15 g	2.5
IRON-EDTA	37.33 g	0.5
1 M $MGSO_4.7H_2O$	246.47 g	1
1 M KH_2PO_4 (PH6)	136.086 g	0.5
Micronutrients		
H_3BO_3	2.86 g	0.5
$MNCL_2.4H_2O$	0.18 g	0.5
$ZNSO_4.7H_2O$	0.22 g	0.5
$CUSO_4.5H_2O$	0.08 g	0.5
$H_2MOO_4.H_2O$	0.02 g	0.5

Morphological and biochemical parameters

At the seedling stage of tomato, data of different parameters of each genotype was recorded after 40 days of transplantation into polythene cups. Data were recorded for morphological parameters such as plant height (cm), number of branches, number of leaves, shoot length (cm), root length (cm), dry root weight (g), dry shoot weight (g), fresh root weight (g), fresh shoot weight (g), fresh plant biomass (g), dry plant biomass (g), dry root/shoot weight ratio (%) and fresh root/shoot weight ratio (%) and biochemical parameters such as cadmium determination of roots (ppm) and cadmium determination of leaves (ppm).

Sample digestion

Each dry mass per plant was measured with the help of a Portable digital balance. For sample digestion, 0.25 g of plant dry sample was measured and placed in 50ml of conical flasks. Nitric acid and perchloric acid in a 3:1 ratio were added with the help of a pipette. Flasks were covered with the help of aluminium foil and left overnight inside the fume hood. Material was heated on the hot plate in the fume hood overnight at 200-250 °C temperature. The volume of each sample was reduced to 1ml. After leaving the samples to cool, 50ml distilled water was added to each sample. For the filtration process of the solution, filter paper was set up in the funnel, and the solution was carefully poured into plastic bottles. At 100 ppm level, calibrated standards were prepared in the form of an aqueous solution. Distilled water was used throughout the process. Cadmium concentrations were recorded by using the apparatus.

Statistical analysis

Collected data were subjected to an analysis of variance (ANOVA) by following the method of Steel et al. (1997) to determine significant differences in parameters among all genotypes. For this purpose, Statistix (Version 12.0) was used to perform the analysis. For comparison of variability, the

coefficient of variation was calculated for all the characters as well. The characters with significant differences were selected for further study. To estimate genetic divergence among twenty different genotypes of tomato, principal component analysis (Pearson, 1901; Sheath & Sokal, 1973), a simple nonparametric method of getting relevant data was performed. Tolerance and non-tolerance mechanisms of each plant per genotype were identified by principal component analysis using XLSTAT (Ver 2023.1).

Results and Discussion

Estimation of variability and mean comparison test

The results of ANOVA for all genotypes (G), both treatments (T) i.e., control and 6 ppm cadmium stress, and their interaction (T×G) between treatment levels of cadmium and all twenty tomato genotypes were illustrated in Table 3. All the genotypes used in the experiment showed highly significant variation from one another. The genotypes were selected based on the best performance under stress levels. Traits under study were shoot length, root length, plant height, fresh root weight, fresh shoot weight, dry root weight, dry shoot weight, Number of branches, Number of leaves, fresh plant biomass, dry plant biomass, fresh root/shoot ratio, dry root/shoot ratio, cadmium concentration in root, shoot and leaves. From the results illustrated in the table it was clear that both treatments i.e., control and stress level (6ppm cadmium) showed highly significant differences for most of the traits including NOB, NOL, DRW, DSW, DPB, FRS, CdCR, CdCL and FRW while all other traits were found non-significant. Dry shoot weight, dry plant biomass, cadmium concentration of root and leaves showed significant interaction of genotypes and cadmium levels while number of branches, number of leaves, dry root weight, fresh plant biomass, dry root/shoot ratio, fresh root/shoot ratio, shoot length, root length, fresh root weight, fresh shoot weight and plant height showed non-significant interaction between treatment and genotypes.

Tukey HSD all-pairwise comparisons test for dry root weight under control and stressed conditions were given in Table 4 and Table 5, respectively. Under treatment 1 (normal) maximum value was shown by genotype Peto 86 for number of branches (7.835) and fresh shoot weight (15.830); genotype 19899 for number of leaves (43.500), fresh root/shoot ratio (38.848), shoot length (45.500), plant height (52.00), cadmium concentration in shoot for (25.00); genotype CLN-3552D for dry root weight (0.905); Cchaus for dry shoot weight (1.925), fresh plant biomass (21.540), dry plant biomass (2.790) and fresh root weight (5.775); genotype 19857 for dry root/shoot ratio (55.065); genotype 19897 for root length (29.165); 19895 for cadmium concentration in roots (76.00) and genotype 19908 for cadmium concentration in leaves (56.00).

In accordance with the findings, genotype Peto 86 for the number of branches (7.750) and number of leaves (43.500); genotype 19857 for dry root weight (1.260), dry shoot weight (1.940), fresh shoot weight (23.125), fresh plant biomass (32.345) and fresh root weight (9.220); CLN-3552D for dry plant biomass (2.425), genotype 19908 for dry root/shoot ratio (53.406), and cadmium concentration in leaves (28.00); genotype 18278 for fresh root/shoot ratio (56.179); genotype 19899 for shoot length (44.500); genotype 19897 for root length (27.170); genotype 17874 for plant height (68.500); genotype 19895 for cadmium concentration in roots (38.00) performed well under stress level (6ppm cadmium level). The results were similar to the findings of root morphological traits (Naciri et al., 2021) such as root diameter, dry matter, root length, etc., and shoot morphological traits (Naciri et al., 2021) such as shoot surface area, dry matter, shoot length, etc., revealed different associations under stress conditions. Results suggested that under cadmium levels, root dry weight and shoot dry weight significantly decreased as the concentration levels of metals increased. Cadmium toxicity and dry root weight (Alves et al., 2020; Tayeb et al., 2019), fresh root/shoot ratio (Gharaibeh et al., 2016) and root length (Okori et al., 2021) had a significant relation under all doses of stress while non-significant association between cadmium toxicity and dry root weight (Okori et al., 2021), dry shoot weight (Gharaibeh et al., 2016; Ahmad et al., 2018), plant fresh biomass (Gharaibeh et al., 2016) that dry plant biomass (Zhao et al., 2016; Naciri et al., 2021), shoot growth (Borges et al., 2018; Okori et al., 2021) and root length (Borges et al., 2018; Ahmad et al., 2018; Tayeb et al., 2019). Total biomass of tomato rootstock decreased when plants were sown under cadmium conditions and the results were matched with the findings of Liu et al. (2016); Ahmad et al. (2018). The variation in dry root/shoot weight was due to different doses of potassium and cadmium (Naciri et al., 2021).

In accordance with our findings, the dry root/shoot weight ratio with response to cadmium levels, significant results were observed (Alves et al., 2020), while non-significant relation was observed by Okori et al. (2021). Root fresh weight and fresh shoot weight (Tayeb et al., 2019) had significant associations with metal stress. So, it was concluded that for this trait increased level of cadmium appeared to be significant for some genotypes while for others it proved to be non-significant. Cadmium (Cd) toxicity in plants leads to a series of physiological changes that negatively affect growth and development, as observed in the tomato genotypes in this study. The cd ions interfere with the uptake of other nutrients by replacing other elements such as calcium, iron and zinc that are vital for the plant's health (Ma et al., 2015). Differential response of root and shoot growth rate under cadmium stress can be explained by the reduction in root tip elongation and interruption in cell metabolism and nutrition process. Moreover, by altering redox homeostasis, Cd causes the generation of ROS, which negatively affects the membranes, proteins and lipids of the plant cells and decreases plant growth (Gharaibeh et al., 2016). In response, some genotypes may employ tolerance mechanisms such as enhanced metal sequestration in vacuoles or cell walls and activating antioxidant defense systems to mitigate oxidative damage (Alves et al., 2020). In addition, changes in root mass or root

structure may decrease the amount of Cd uptake and alleviate the metal's toxic effects (Zhao et al., 2016). These results imply that the mechanisms of cadmium stress tolerance in tomato genotypes involve metal tolerance, nutrient balance, and oxidative stress defense.

The findings of this study have important implications for agriculture in regions with cadmium-contaminated soils. The practical application of this study involves the development of usable resources to identify the cadmium-tolerant tomato genotypes that would enhance crop production and food security in the areas of interest. Introducing these genotypes to breeding programs may reduce cadmium's impact on yields and nutritional values.

This study was conducted under controlled conditions, which may not fully reflect field environments. The research also focused on a limited range of genotypes and cadmium concentrations. Future studies should explore a broader range of genotypes, cadmium levels, and the molecular mechanisms of cadmium tolerance to understand better how tomatoes adapt to heavy metal stress. Future research on cadmium (Cd) tolerance in tomato genotypes should evaluate for metal-tolerant protein genes and Glutathione S-transferases responsible for metal channel and detoxification. Biochemical assays could also determine antioxidant enzyme activity such as superoxide dismutase, catalase, and peroxidases, which help attenuate the toxic effect of Cd. Moreover, mechanistic studies on metal uptake using radio-labelled Cd and histochemical studies to map Cd in plant tissues will assist in isolating genotypes with low Cd toxicity and a high ability to compartmentalize Cd. These approaches shall help improve understanding the Cd tolerance and generation of tolerant tomato varieties.

Table 3 Analysis of variance for morphophysiological traits of tomato (*L. esculentum* L.) genotypes

Sources of Variance (SOV)	Treatment (T)	Genotypes (G)	T × G	Error
	DF = 1	DF = 19	DF = 19	DF = 40
Number of branches	5.698	3.552	$0.334^{N.S}$	0.611
Number of leaves	141.406*	125.38	$17.329^{N.S}$	21.337
Dry root weigh	0.218	0.124	$0.048^{N.S}$	0.029
Dry shoot weight	0.697	0.339	0.149	0.059
Fresh plant biomass	$5.156^{N.S}$	52.907	$21.455^{N.S}$	13.082
Dry plant biomass	1.696	0.788	0.346	0.143
Dry root/shoot ratio	$201.473^{N.S}$	263.974	$70.254^{N.S}$	97.693
Fresh root/shoot ratio	828.417	171.343^{*}	$34.796^{N.S}$	86.292
Shoot length	$8.020^{N.S}$	74.136	$10.357^{N.S}$	19.202
Root length	$4.675^{N.S}$	28.898	$10.809^{N.S}$	10.147
Fresh root weight	11.430*	5.807	$2.183^{N.S}$	2.236
Fresh shoot weight	$1.232^{N.S}$	27.364	$10.715^{N.S}$	6.304
Plant height	$24.920^{N.S}$	81.955*	$27.883^{N.S}$	38.459
Cadmium concentration of root	63845	205.5	205.5	25.00
Cadmium concentration of leaves	27306.1	61.4	61.4	25.500

Significant = * ($p<0.05$); highly significant = ($p<0.01$); non-significant = N.S ($p>0.05$)

Principal component analysis

Principal component analysis is a resourceful tool for determining variation patterns and population structure present between genotypes. The more extensive data could be converted into a smaller set consisting of all information of larger sets. This makes it easy to analyze small data except larger ones. PC reduces the number of variations and conserves all information. It is used to extract more information from data (Nazir et al., 2017). Principal component analysis was used to study the mean data of traits. The genetic divergence between 20 genotypes of tomato was determined using XLSTAT. Principal component analysis provides information about standard deviation, mean, maximum and minimum values of factors contributing, Eigenvectors, Scree plot, Biplot, Scatter plot and Eigenvalue. The diverse values of summary statistics, i.e., standard deviation, mean, maximum and minimum values for normal and stress treatment, are shown in Table 6 and Table 8, respectively. The eigenvalues of principle components 1, 2 and 3 at the control condition (Table 7) were significant as all components gave values (6.013, 3.441, and 1.928, respectively) more than 1. Eigenvectors, Eigenvalues, and variability explained by the first 3 principal components for 13 characters in 20 genotypes of tomato under stress (6 ppm cadmium) are given in Table 9.

Scatter plot

The scatter plot provides valuable insights into the genetic diversity of tomato genotypes. Those positioned further from the origin demonstrate more significant genetic variability, while those closer to the origin share similar properties. As depicted in Fig. 1, genotypes 19899, 19865, and Naqeeb show the most diverse variation under control conditions, positioning them further from the origin. In contrast, Money Maker, 17074, and 17868 are clustered closely together, indicating their genetic similarity. CLN-3552D and Cchaus share common properties and are positioned close to each other,

while 19897 and 19895 are grouped in a separate cluster. Under PC1, Cchaus and CLN-3552D perform remarkably well, while 19899 emerges as the top performer under PC2. Unfortunately, Naqeeb and 19908 underperformed in PC2, with 17266 and 19865 lagging as poor performers (Table 9). In Fig. 2, stress conditions are evaluated, and it is revealed that Kanatoo, 19895, and Money Maker share similar properties, forming a tight cluster.

In contrast, Naqeeb, 18278, and 17874 show significant genetic differences, positioning them further from the origin. Under PC1, 19857 stands out as the top performer, while Naqeeb disappoints. In contrast, 19899 outperforms under PC2, with 18278 lagging as a poor performer. These findings shed light on the genetic diversity of tomato genotypes, identifying top performers under different conditions that could benefit future breeding programs.

Biplot

The biplot is a graphical tool that characterizes variables via vector length. Essentially, the longer the vector, the more significant the contribution of that variable to the overall variation. When traits are farther from the origin, it indicates greater genetic diversity and fewer similarities, while traits closer to the origin reveal maximum similarities. The line that joins the character towards the origin is known as a trait vector (Mahmood et al., 2022). Meanwhile, the angle that shows the association between all parameters is termed the cosine angle. Parameters with more than 90 degrees angle correlate negatively, while parameters with less than 90 degrees correlate positively. Under control conditions, genotypes CLN-3552D and Cchaus displayed positive performance and were located closest to the origin, while genotype Naqeeb and 19897 showed negative performance (Fig. 3). Conversely, under stress conditions, genotype 19857 demonstrated positive performance. In contrast, genotype Naqeeb displayed negative performance (Fig. 4).

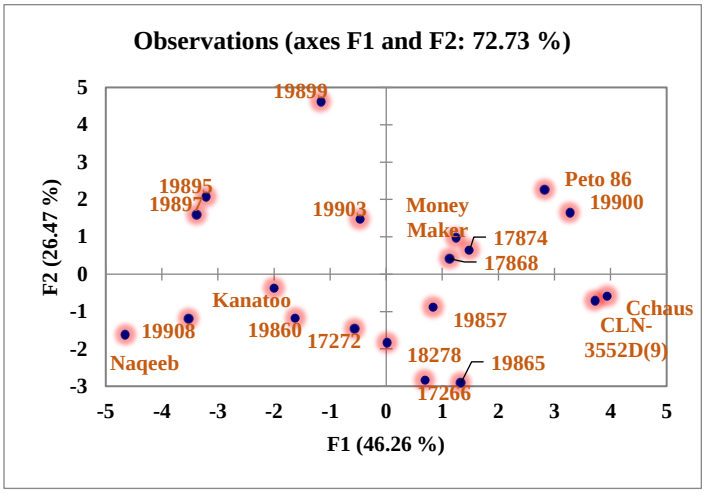

Fig. 1 Two-dimensional orientation of tomato germplasm on principal components (1 and 2) for the data collected under
control

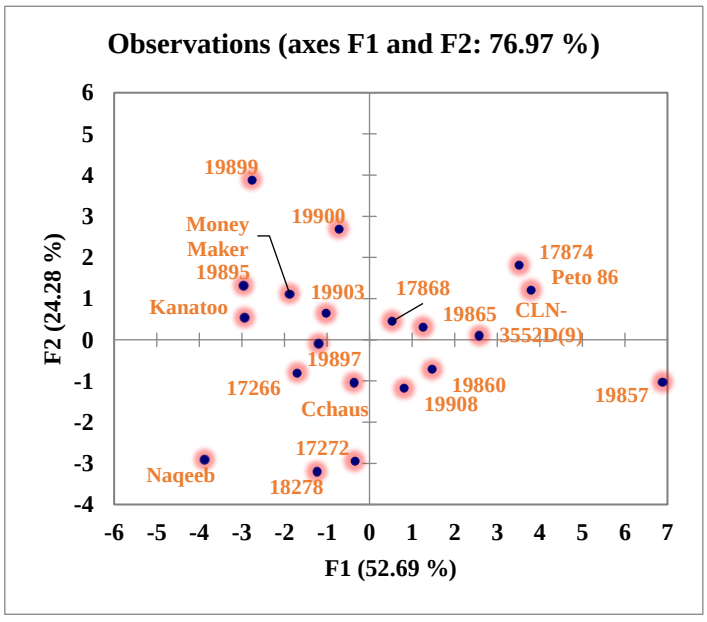

Fig. 2 Two-dimensional orientation of tomato germplasm on principal components (1 and 2) for the data collected under stress conditions (6 ppm cadmium)

Table 4 Tukey HSD all-pairwise comparisons test for dry root weight under control and stress conditions

Sr. No.	Genotypes	NOB	NOL	DRW	DSW	FPB	DPB	DRSR	FRSR	SL	RL	FRW	FSW	PH	CDCR	CDCL
1	17868	6.34	35.34	0.68	1.62 abcd	18.76	2.30 abcd	41.99	31.71	37.67	25.83	4.62	14.14	63.50	0.00 h	0.00 c
2	CLN-3552D	7.33	39.17	0.91	1.86 abc	20.98	2.76 ab	49.08	35.12	36.50	22.34	5.49	15.49	58.83	0.00 h	0.00 c
3	Kanatoo	5.50	30.33	0.46	1.41 bcd	15.70	1.86 bcd	32.34	24.42	34.00	18.00	2.99	12.70	52.00	0.00 h	0.00 c
4	17266	4.34	22.84	0.72	1.74 abcd	19.06	2.46 abcd	42.40	30.61	33.50	20.67	4.32	14.74	54.17	0.00 h	0.00 c
5	17272	5.67	31.34	0.65	1.46 abcd	15.86	2.11 bcd	44.71	32.43	35.50	18.67	3.91	11.96	54.17	0.00 h	0.00 c
6	18278	6.00	33.67	0.70	1.42 bcd	17.28	2.11 bcd	49.02	38.44	33.00	23.33	4.73	12.55	56.34	0.00 h	0.00 c
7	Naqeeb	4.50	26.00	0.42	1.07 bcd	12.55	1.49 bcd	38.29	35.13	28.34	28.50	3.20	9.35	56.83	0.00 h	0.00 c
8	19908	6.17	35.00	0.43	1.04 bcd	13.17	1.47 bcd	41.48	38.89	30.17	22.67	3.67	9.50	52.83	0.00 h	0.00 c
9	19903	7.50	40.17	0.54	1.56 abcd	15.96	2.10 bcd	34.81	35.43	34.84	27.67	4.19	11.77	62.50	0.00 h	0.00 c
10	19857	6.84	37.34	0.77	1.41 bcd	17.69	2.18 abcd	55.07	31.99	35.50	21.17	4.24	13.45	56.67	0.00 h	0.00 c
11	19860	5.84	33.17	0.60	1.33 bcd	14.65	1.92 bcd	46.02	30.13	33.67	19.17	3.31	11.34	52.84	0.00 h	0.00 c
12	17874	7.17	40.17	0.69	1.62 abcd	18.99	2.30 abcd	42.06	25.67	37.50	18.67	4.03	14.96	56.17	0.00 h	0.00 c
13	19865	5.83	32.50	0.84	1.57 abcd	18.21	2.41 abcd	53.81	37.16	33.17	19.67	4.99	13.22	52.86	0.00 h	0.00 c
14	19895	6.50	35.50	0.38	1.29 bcd	12.90	1.67 bcd	29.61	20.89	36.34	21.50	2.22	10.68	57.84	0.00 h	0.00 c
15	19897	6.83	38.00	0.48	1.09 bcd	11.73	1.57 bcd	44.48	32.07	35.33	29.17	2.90	8.84	64.50	0.00 h	0.00 c
16	19899	7.50	43.50	0.47	1.29 bcd	14.57	1.76 bcd	36.13	22.32	45.50	24.84	2.63	11.94	70.34	0.00 h	0.00 c
17	19900	7.33	40.00	0.77	1.89 ab	19.63	2.65 abc	40.56	30.75	44.67	20.00	4.66	14.98	64.67	0.00 h	0.00 c
18	Peto 86	7.67	42.17	0.67	1.87 ab	20.51	2.53 abcd	34.81	27.27	41.17	22.34	4.68	15.83	63.50	0.00 h	0.00 c
19	Money Maker	5.84	32.84	0.58	1.80 abcd	19.78	2.38 abcd	31.97	24.26	39.50	21.34	3.88	15.90	60.84	0.00 h	0.00 c
20	Cchaus	6.50	35.84	0.87	1.93 ab	21.54	2.79 ab	44.91	35.96	39.83	22.33	5.78	15.77	62.17	0.00 h	0.00 c

NOB (Number of branches); **NOL** (Number of leaves); **DRW** (Dry root weight); **DSW** (Dry shoot weight); **FPB** (Fresh plant biomass); **DPB** (Dry plant biomass); **DRSR** (Dry root/shoot weight ratio; **FRSR** (fresh root/shoot weight ratio); **SL** (Shoot length); **RL** (Root length); **FRW** (Fresh root weight); **FSW** (Fresh shoot weight); **PH** (Plant height); **CdCR** (Cadmium concentration of roots); **CdCL** (Cadmium determination of leaves)

Table 5 Tukey HSD all-pairwise comparisons test for dry root weight under control and stress conditions

Sr. No.	Genotypes	NOB	NOL	DRW	DSW	FPB	DPB	DRSR	FRSR	SL	RL	FRW	FSW	PH	CDCR	CDCL
1	17868	5.84	34.67	0.51	1.35 bcd	17.75	1.86 bcd	37.61	40.52	37.50	25.17	5.09	12.66	62.67	55.00 bcdef	41.00 ab
2	CLN-3552D	5.84	34.67	0.51	1.35 bcd	17.75	61.8 bcd	37.61	40.52	37.50	25.17	5.09	12.66	62.67	55.00 bcdef	41.00 ab
3	Kanatoo	6.84	38.17	0.72	1.71 abcd	21.53	2.43 abcd	42.84	40.47	36.17	25.00	6.19	15.34	61.17	47.00 def	37.00 ab
4	17266	4.34	25.84	0.25	1.07 bcd	14.53	1.31 bcd	22.69	26.55	34.34	19.00	3.05	11.48	53.34	65.00 abcd	45.00 ab
5	17272	4.33	24.67	0.39	1.26 bcd	16.40	1.64 bcd	29.90	36.24	32.67	21.00	4.40	12.00	53.67	62.00 abcde	35.00 ab
6	18278	4.83	26.84	0.65	1.26 bcd	17.39	1.91 bcd	49.87	50.09	30.00	20.67	5.87	11.53	50.67	44.00 efg	42.00 ab
7	Naqeeb	4.67	24.84	0.59	1.03 bcd	14.43	1.62 bcd	52.55	56.18	30.50	21.83	5.32	9.12	52.33	26.00 g	38.00 ab
8	19908	4.00	17.17	0.34	0.81 d	11.07	1.15 cd	41.61	42.67	24.67	25.34	3.33	7.74	50.00	75.00 ab	31.00 b
9	19903	7.00	39.00	0.63	1.20 bcd	17.27	1.83 bcd	53.41	49.17	32.34	23.17	5.59	11.68	55.50	57.00 abcde	56.00 a
10	19857	6.50	38.83	0.40	1.19 bcd	14.93	1.59 bcd	36.09	32.75	32.84	21.00	3.49	11.44	53.84	59.00 abcde	26.00 b
11	19860	6.34	36.17	1.26	2.47 a	32.35	3.73 a	50.77	40.33	40.84	19.00	9.22	23.13	59.84	43.00 efg	47.00 ab
12	17874	5.50	31.34	0.78	1.55 abcd	21.47	2.33 abcd	50.00	34.56	37.67	17.34	5.45	16.03	55.00	72.00 abc	29.00 b
13	19865	6.83	39.50	0.60	1.87 ab	24.59	2.47 abcd	32.10	37.13	41.67	26.84	6.67	17.92	68.50	69.00 abc	41.00 ab
14	19895	6.33	36.17	0.61	1.48 abcd	20.08	2.09 bcd	40.57	36.82	36.83	21.83	5.39	14.69	58.67	43.00 efg	31.00 b
15	19897	6.17	34.33	0.26	0.85 cd	11.24	1.10 cd	28.61	28.63	34.50	19.33	2.52	8.72	53.84	76.00 a	46.00 ab
16	19899	6.00	34.33	0.48	0.92 bcd	12.51	1.40 bcd	51.81	37.42	33.67	27.17	3.38	9.13	60.84	53.00 cdef	37.00 ab
17	19900	6.50	35.17	0.20	0.80 d	9.87	0.99 d	24.09	21.48	44.50	19.50	1.74	8.13	64.00	75.00 ab	27.00 b
18	Peto 86	6.00	33.67	0.33	1.32 bcd	14.99	1.65 bcd	22.83	30.29	43.50	20.67	3.56	11.43	64.17	61.00 abcde	32.00 b
19	Money Maker	7.84	44.83	0.75	1.75 abcd	22.85	2.50 abcd	42.92	42.01	40.00	25.34	6.76	16.09	65.34	36.00 fg	38.00 ab
20	Cchaus	5.17	30.00	0.29	1.23 bcd	16.50	1.52 bcd	22.94	25.47	35.84	17.67	3.37	13.14	53.50	69.00 abc	33.00 b

NOB (Number of branches); **NOL** (Number of leaves); **DRW** (Dry root weight); **DSW** (Dry shoot weight); **FPB** (Fresh plant biomass); **DPB** (Dry plant biomass); **DRSR** (Dry root/shoot weight ratio; **FRSR** (fresh root/shoot weight ratio); **SL** (Shoot length); **RL** (Root length); **FRW** (Fresh root weight); **FSW** (Fresh shoot weight); **PH** (Plant height); **CdCR** (Cadmium concentration of roots); **CdCL** (Cadmium determination of leaves)

Table 6 Summary statistics of tomato genotypes under control condition

Variables	Minimum	Maximum	Mean	St. Deviation
SL	28.333	45.500	36.283	4.295
RL	18.00	29.166	22.391	3.299
PH	52.00	17.333	58.675	5.085
FRW	2.215	5.775	4.019	0.922
FSW	8.840	15.900	12.954	2.266
DRW	0.380	0.905	0.629	0.157
DSW	1.035	1.925	1.509	0.279
NOB	4.333	7.666	6.358	0.949
NOL	22.833	43.00	35.241	5.201
FPB	11.730	21.540	16.973	3.002
DPB	1.465	2.790	2.139	0.410
FRSR	20.888	38.848	31.029	5.367
DRSR	29.611	55.065	41.676	7.060

SL= Shoot length, **RL=** Root length, **PH=** Plant height**, FRW=** Fresh root weight, **FSW=** Fresh shoot weight, **DRW=** Dry root weight, **DSW=** Dry shoot weight, **NOB=** Number of branches, **NOL=** Number of leaves, **FPB=** Fresh plant biomass, **DPB=** Dry plant biomass, **FRSR=** Fresh root/shoot ratio, **DRSR=** Dry root/shoot ratio

Table 7 Eigenvectors, Eigenvalues and variability explained by first 3 principal components for 13 characters in 20 genotypes of tomato under control

Traits	PC1	PC2	PC3
SL	0.233	0.399	-0.088
RL	-0.144	0.157	0.476
PH	0.103	0.439	0.234
FRW	0.343	-0.207	0.220
FSW	0.379	0.008	-0.220
DRW	0.359	-0.199	0.138
DSW	0.383	0.015	-0.159
FPB	0.394	-0.059	-0.097
DPB	0.399	-0.065	-0.055
FRSR	0.032	-0.341	0.509
DRSR	0.119	-0.322	0.373
NOB	0.163	0.384	0.278
NOL	0.136	0.404	0.281
Eigenvalue	6.013	3.441	1.928
Variability (%)	46.259	26.469	14.837
Cumulative variability (%)	46.259	72.729	87.566

SL= Shoot length, **RL=** Root length, **PH=** Plant height**, FRW=** Fresh root weight, **FSW=** Fresh shoot weight, **DRW=** Dry root weight, **DSW=** Dry shoot weight, **NOB=** Number of branches, **NOL=** Number of leaves, **FPB=** Fresh plant biomass, **DPB=** Dry plant biomass, **FRSR=** Fresh root/shoot ratio, **DRSR=** Dry root/shoot ratio

Table 8 Summary statistics of tomato genotypes under stress condition (6ppm cadmium)

Variables	Minimum	Maximum	Mean	Standard deviation
SL	24.666	44.500	35.650	4.877
RL	17.333	27.166	21.908	2.995
PH	50.00	68.500	57.558	5.389
FRW	1.740	9.220	4.775	1.762
FSW	7.740	23.125	12.706	3.728
DRW	0.195	1.260	0.524	0.247
DSW	0.795	2.470	1.323	0.407
NOB	4.00	7.833	5.825	1.020
NOL	17.166	44.833	32.583	6.656
FPB	9.870	32.345	17.481	5.307
DPB	0.990	3.730	1.847	0.631
FRSR	21.478	56.179	37.465	8.617
DRSR	22.687	53.406	38.502	10.829
CDCR	26.00	76.00	56.500	14.336
CDCL	26.00	56.00	36.950	7.837

SL= Shoot length, **RL=** Root length, **PH=** Plant height**, FRW=** Fresh root weight, **FSW=** Fresh shoot weight, **DRW=** Dry root weight, **DSW=** Dry shoot weight, **NOB=** Number of branches, **NOL=** Number of leaves, **FPB=** Fresh plant biomass, **DPB=** Dry plant biomass, **FRSR=** Fresh root/shoot ratio, **DRSR=** Dry root/shoot ratio, **CdCR=** Cadmium concentration in root**s; CdcL=** Cadmium concentration in leaves

Table 9 Eigenvectors, Eigenvalues and variability explained by first 3 principal components for 13 characters in 20 genotypes of tomato under stress (6 ppm cadmium)

Traits	PC1	PC2	PC3
SL	0.122	0.460	-0.029
RL	0.086	-0.070	0.586
PH	0.158	0.377	0.299
FRW	0.349	-0.104	-0.083
FSW	0.322	0.117	-0.269
DRW	0.340	-0.101	-0.109
DSW	0.337	0.092	-0.212
FPB	0.342	0.047	-0.217
DPB	0.350	0.020	-0.180
FRSR	0.164	-0.408	0.222
DRSR	0.198	-0.330	0.181
NOB	0.197	0.295	0.357
NOL	0.201	0.313	0.311
CDCR	-0.209	0.234	-0.152
CDCL	0.123	-0.099	0.119
Eigenvalue	7.539	3.647	1.859
Variability (%)	47.122	22.799	11.623
Cumulative variability (%)	47.122	69.921	81.545

SL= Shoot length, **RL=** Root length, **PH=** Plant height**, FRW=** Fresh root weight, **FSW=** Fresh shoot weight, **DRW=** Dry root weight, **DSW=** Dry shoot weight, **NOB=** Number of branches, **NOL=** Number of leaves, **FPB=** Fresh plant biomass, **DPB=** Dry plant biomass, **FRSR=** Fresh root/shoot ratio, **DRSR=** Dry root/shoot ratio, **CdCR=** Cadmium concentration in root**s; CdcL=** Cadmium concentration in leaves

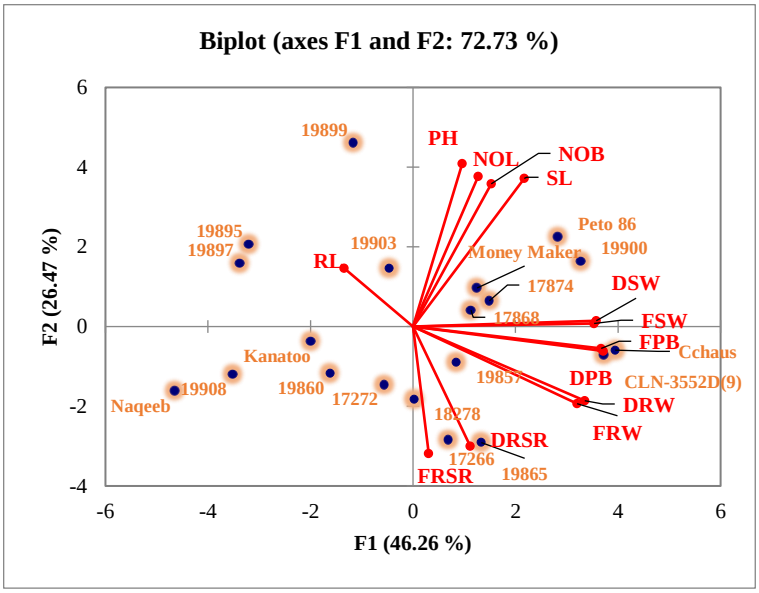

Fig. 3 Principal component biplot of 20 tomato genotypes under control condition (0 ppm)
SL= Shoot length, **RL**= Root length, **PH**= Plant height**, FRW**= Fresh root weight, **FSW**= Fresh shoot weight, **DRW**= Dry root weight, **DSW**= Dry shoot weight, **NOB**= Number of branches, **NOL**= Number of leaves, **FPB**= Fresh plant biomass, **DPB**= Dry plant biomass, **FRSR**= Fresh root/shoot ratio, **DRSR**= Dry root/shoot ratio

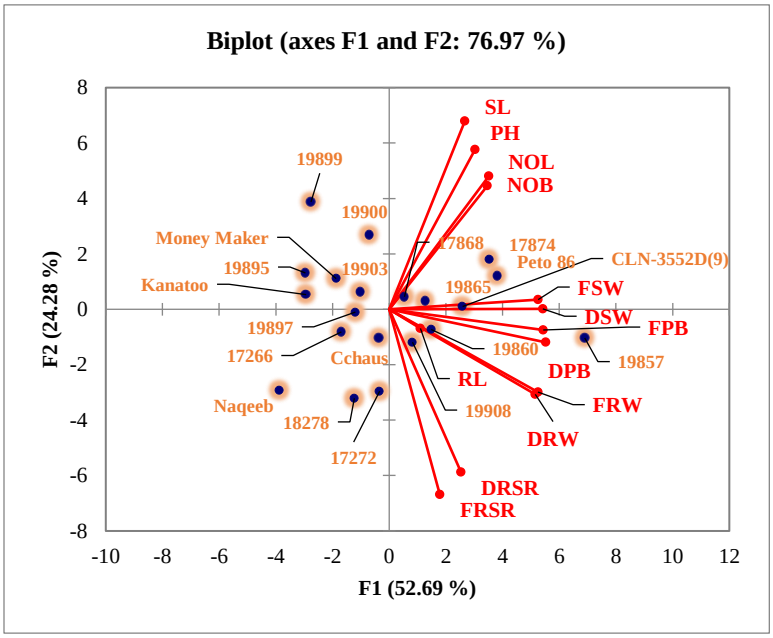

Fig. 4 Principal component biplot of 20 tomato genotypes under stress condition (6ppm cadmium)
SL= Shoot length, **RL**= Root length, **PH**= Plant height**, FRW**= Fresh root weight, **FSW**= Fresh shoot weight, **DRW**= Dry root weight, **DSW**= Dry shoot weight, **NOB**= Number of branches, **NOL**= Number of leaves, **FPB**= Fresh plant biomass, **DPB**= Dry plant biomass, **FRSR**= Fresh root/shoot ratio, **DRSR**= Dry root/shoot ratio

Conclusion

This study assessed the genetic diversity and growth performance of 20 tomato genotypes under cadmium stress. The results revealed significant differences among the genotypes, highlighting the existence of Cd-tolerant tomato varieties with potential for breeding programs. Genotypes 17868, 17874, and Money Maker performed well under control conditions, while Naqeeb and Cchaus showed poor performance. Under Cd stress, genotypes 19860, 17868, and 19865 exhibited favorable performance, whereas Naqeeb and 19899 performed poorly. These findings contribute valuable insights into identifying Cd-tolerant tomato genotypes, providing potential improvements for tomato cultivation in Pakistan. The study emphasizes the importance of screening for Cd tolerance in crop plants and selecting appropriate genotypes for cultivation in Cd-contaminated soils. Further research and breeding efforts can focus on these Cd-tolerant genotypes to develop improved tomato varieties that can withstand the adverse effects of heavy metal stress, ultimately enhancing agricultural productivity and food security.

Additionally, integrating these tolerant varieties into sustainable farming practices could help mitigate the impact of Cd contamination on agricultural productivity. In the long run, the generation of tomato plants that have Cd tolerance can improve food security in areas with soil contamination problems and produce healthy crops that are resistant to heavy metal stress. Further studies should be directed to identifying the molecular and biochemical basis of Cd tolerance in these genotypes to advance understanding of how these lines can

respond to heavy metal stress and generate better-adapted genotypes.

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