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Research Article/Araştırma Makalesi

Characterization of pepper (*Capsicum annuum* L.) genotypes for zinc stress tolerance based on morphological traits

Çinko stresi altında biber (Capsicum annuum L.) genotiplerinin morfolojik tepkilerinin karakterizasyonu

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ABSTRACT

Zinc (Zn) stress negatively affects plant growth and physiology; however, genotypic variation influences the degree of tolerance. In this study, 28 genetically distinct *Capsicum annuum* genotypes were evaluated under hydroponic Zn stress (5 mM $ZnCl_2$) and control conditions. Morphological and physiological traits, including SPAD values, leaf number, shoot and root length, stem diameter, leaf dimensions, and biomass (fresh/dry weight), were measured. Genotypes P114, P82, and P116 exhibited minimal SPAD reductions (<5%), whereas P41, P161, and P159 showed >30% declines. P120, P45, and P171 maintained leaf number under stress, while root elongation increased in P164 and P50 by over 70%. Biomass retention was highest in P31, P164, and P161, while lowest in P173 and P49. Principal Coordinate Analysis (PCoA) identified P159, P164, P114, and P46 as Zn-tolerant genotypes. Strong correlations were found between fresh and dry weight (r = 0.90), root length and dry weight (r = 0.55), and leaf width and fresh weight (r = 0.46). These findings reveal key physiological traits linked to Zn tolerance and provide valuable insights for breeding stress-resilient pepper cultivars.

Key Words: *Capsicum sp.*, zinc stress, genotype variation, PCoA, stress tolerance

ÖZ

Çinko (Zn) stresi, bitki büyümesini ve fizyolojisini olumsuz etkilemektedir; ancak, genotipik varyasyon tolerans düzeyini değiştirebilmektedir. Bu çalışmada, genetik olarak farklı 28 *Capsicum annuum* genotipi, hidroponik Zn stresi (5 mM $ZnCl_2$) ve kontrol koşulları altında değerlendirilmiştir. SPAD değerleri, yaprak sayısı, sürgün ve kök uzunluğu, gövde çapı, yaprak boyutları ve biyokütle (yaş/kuru ağırlık) gibi morfolojik ve fizyolojik özellikler ölçülmüştür. Genotipler P114, P82 ve P116, <%5 SPAD azalması ile minimum düşüş sergilerken; P41, P161 ve P159 >%30 azalma göstermiştir. P120, P45 ve P171, stres altında yaprak sayısını korurken, P164 ve P50 genotiplerinde kök uzaması %70'in üzerinde artış göstermiştir. Biyokütle korunumu en yüksek P31, P164 ve P161'de; en düşük ise P173 ve P49'da gözlenmiştir. Temel Koordinat Analizi (PCoA), P159, P164, P114 ve P46 genotiplerini Zn-tolerant (çinko toleranslı) olarak tanımlamıştır. Yaş ve kuru ağırlık arasında (r = 0.90), kök uzunluğu ile kuru ağırlık arasında (r =0.55) ve yaprak genişliği ile yaş ağırlık arasında (r = 0.46) güçlü korelasyonlar bulunmuştur. Bu bulgular, Zn toleransı ile ilişkili temel fizyolojik özellikleri ortaya koymakta ve stres dayanımlı biber çeşitlerinin ıslahı için değerli bilgiler sunmaktadır.

Anahtar Kelimeler: *Capsicum* sp., çinko stresi, genotip varyasyonu, PCoA, stres toleransı

Introduction

Heavy metal (HM) toxicity is among the most severe abiotic stressors affecting plant health and agricultural productivity. Industrialization, mining, urbanization, and excessive use of agrochemicals have intensified the accumulation of toxic metals such as cadmium (Cd), lead (Pb), arsenic (As), and zinc (Zn) in soil and water ecosystems (Briffa et al., 2020). While zinc is an essential micronutrient required for plant growth, functioning as a cofactor for many enzymes and contributing to chlorophyll synthesis, protein metabolism, and hormonal regulation (Escudero-Almanza et al., 2012), its excess can induce phytotoxicity. Elevated Zn levels disrupt cellular redox balance, inhibit photosynthesis, and limit root elongation, ultimately reducing plant biomass and yield (Ul-Hassan et al., 2017; Mukhopadhyay et al., 2013).

Zinc toxicity in plants typically arises when concentrations exceed species-specific thresholds. In *C. annuum*, Zn concentrations above 200 µM in hydroponic systems or 100–300 mg/kg in soil can trigger stress responses (Moreira et al., 2018). The resulting symptoms include chlorosis, stunted growth, oxidative damage, and impaired nutrient uptake. Understanding the range of physiological responses across pepper genotypes is essential for developing cultivars with enhanced metal tolerance.

Pepper (*C. annuum* L.) is one of the most widely cultivated horticultural crops, valued for its nutritional, medicinal, and economic attributes. Globally, it occupies over 2 million hectares with an annual yield surpassing 38 million tons (FAOSTAT, 2023). Türkiye ranks among the top producers and harbors a rich diversity of pepper landraces and breeding lines. However, the potential of this germplasm to withstand Zn stress remains largely unexplored. Although several studies have reported the general impact of Zn on pepper growth and physiology, they often focus on a single cultivar or specific tissue responses (e.g., vascular structure changes or gene expression profiles) (Farhazadi & Arbabian, 2024), without integrating whole-plant morphological parameters across diverse genetic backgrounds. Considering its dietary importance, wide cultivation area, and nutritional value, especially in developing countries, pepper is a strategic crop for sustainable food systems. Moreover, the accumulation of Zn in pepper fruits poses a potential health risk. Therefore, understanding Zn stress responses in this crop is not only agronomically important but also critical for food safety and phytoremediation efforts.

Recent advances in stress physiology suggest that genotypic screening under hydroponic systems allows controlled analysis of responses to Zn stress, facilitating identification of traits such as chlorophyll retention, biomass stability, and root elongation. Unlike prior studies that focused on single cultivars or isolated physiological traits, this study integrates whole-plant morphological and physiological assessments across 28 genetically diverse pepper genotypes under standardized Zn stress conditions.

This study addresses this knowledge gap by evaluating 28 genetically distinct *C. annuum* genotypes from various Turkish provinces under hydroponic Zn stress (5 mM $ZnCl_2$). Morphological and physiological traits were quantified to assess inter-genotypic variation in stress responses. The objectives were to (i) characterize Zn-induced morphological and physiological changes, (ii) identify Zn-tolerant and Zn-sensitive genotypes, and (iii) provide insights for breeding programs targeting metal-contaminated environments. By integrating multivariate statistical analysis with detailed phenotypic screening, this research offers a comprehensive framework for selecting stress-resilient genotypes and contributes novel findings to the field of heavy metal tolerance in horticultural crops.

Material and Methods

Plant material and growth conditions

Twenty-eight S4-stage pepper (*C. annuum*) genotypes were collected from Hatay, Kayseri, Kahramanmaraş, Muğla, Antalya, Şanlıurfa, and Kilis (Table 1). Seeds were sterilized in 5% sodium hypochlorite for 10 minutes, rinsed with distilled water, and sown in organic-rich soil (54% organic matter, pH 5.5–6.8, Mixflor) in a greenhouse maintained at 25±2°C (day) and 20±2°C (night) under a 16 h light/8 h dark photoperiod, with a light intensity of ~300 µmol m^{-2} s^{-1}. After seedlings reached approximately 15 cm in height, they were transferred to a hydroponic system containing 10% Hoagland nutrient solution (pH 6.2) and acclimated for 7 days. Zn stress was imposed by supplementing the solution with 5 mM $ZnCl_2$, while control plants received no additional Zn. The concentration was selected based on Zn toxicity thresholds reported by Moreira et al. (2018) and to ensure consistent physiological stress without complete growth inhibition. Each genotype was evaluated in a randomized split-plot design with Zn treatment as the main plot and genotype as the sub-plot. Three biological replicates were used for each genotype, and each replicate consisted of 10 individual plants, totaling 30 plants per genotype per treatment.

Table 1. Pepper Genotypes and Their Origins

No	Genotyp Number	Origin	No	Genotyp Number	Origin
1	P29	Hatay	15	P76	Kayseri
2	P31	Hatay	16	P82	Kayseri
3	P41	Hatay	17	P114	Kayseri
4	P45	Hatay	18	P116	Kayseri
5	P46	Kahramanmaraş	19	P120	Kayseri
6	P49	Kahramanmaraş	20	P143	Muğla
7	P50	Kahramanmaraş	21	P159	Muğla
8	P52	Kahramanmaraş	22	P161	Muğla
9	P61	Kahramanmaraş	23	P163	Muğla
10	P63	Şanlıurfa	24	P164	Antalya
11	P64	Şanlıurfa	25	P165	Antalya
12	P68	Şanlıurfa	26	P166	Antalya
13	P69	Şanlıurfa	27	P171	Kilis
14	P71	Kayseri	28	P173	Kilis

Growth and physiological parameters

At the end of the experimental period, plants were harvested and separated into leaves, stems, and roots. Morphological parameters measured included root length, shoot length, stem diameter, leaf width and length, and number of leaves per plant. Fresh weight was measured immediately after harvest, and dry weight was determined by drying the plant samples in a forced-air oven at 65 °C for 48 h (Başak et al., 2025). Chlorophyll content was estimated using a SPAD-502 chlorophyll meter (Konica Minolta, Japan). SPAD values were recorded from the fourth fully expanded leaf on six randomly selected plants per treatment, with readings taken at the same time of day to minimize diurnal variation.

Statistical analysis

All data were analyzed using analysis of variance (ANOVA), and treatment means were compared using Tukey's Honestly Significant Difference (HSD) test at a 5% significance level ($p < 0.05$). Statistical analysis was conducted using SPSS version 17. Results are expressed as mean ± standard error (SE).

Results and Discussion

Morphological and physiological responses of 28 *C. annuum* genotypes to zinc (Zn) stress (5 mM $ZnCl_2$) were evaluated across key traits, including chlorophyll content (SPAD), shoot and root growth, leaf development, and biomass accumulation. The data revealed substantial inter-genotypic variation under Zn stress, which reflects differential tolerance strategies. This diversity is critical for identifying candidates for breeding programs targeting Zn-contaminated soils.

P116 (47.13%) and P163 (46.53%) maintained the highest SPAD values under stress ($p < 0.05$), indicating preserved chlorophyll content and photosynthetic capacity. In contrast, genotypes such as P41 (22.3%) and P161 (28.8%) exhibited severe chlorophyll degradation, suggesting increased susceptibility. Notably, P114 and P82 showed minimal SPAD change (<5%), highlighting their potential tolerance (Table 2). The variation in SPAD responses among genotypes indicates potential genotypic differences in Zn detoxification or compartmentalization mechanisms. Leaf number were also reduced by Zn exposure. P82 and P159 maintained significantly higher leaf counts under Zn stress (20.67 and 19.67, respectively; $p < 0.05$) compared to sensitive genotypes like P116 (9.67) (Table 2). indicating impaired vegetative development (Table 2). In general, genotypes with higher SPAD values also exhibited better leaf retention, suggesting a link between chlorophyll preservation and vegetative resilience. However, exceptions such as P159 (moderate SPAD but high leaf number) imply compensatory mechanisms at the whole-plant level.

Shoot elongation decreased in most genotypes under Zn stress, though with varying severity. P171 (38 cm) and P164 (30 cm) sustained shoot length close to control levels, whereas P31 (15.5 cm) and P45 (16.33 cm) experienced more than 30% reduction. Interestingly, P171 showed a paradoxical increase in shoot length under stress (+9.6% $p < 0.05$), possibly indicating a hormetic effect or efficient internal detoxification (Table 2). Such patterns align with previous findings in metal-tolerant cultivars of tomato and rice, where low-level stress triggers elongation via hormonal modulation. Root length showed unexpected trends. While Zn stress typically inhibits root development, genotypes like P50 (26.67 cm), P45 (24.67 cm), and P164 (23.5 cm) exhibited increased elongation compared to controls, suggesting plasticity in root architecture. This may involve upregulation of genes controlling root elongation and selective ion transport. In contrast, P173 (10.83 cm), P64 (11.67 cm), and P46 (12.1 cm) demonstrated strong reductions, indicative of toxic ion accumulation or cellular damage at the root apical meristem (Table 3).

Table 2. Effects of zinc (Zn) stress on physiological and morphological traits of pepper genotypes: SPAD value, leaf number, and shoot length

Genotypes	SPAD value (%)		Leaf Number		Shoot Length (cm)	
	Control	Zn Stress	Control	Zn Stress	Control	Zn Stress
P29	44.23±4.22a-c	41.57±2.34a-e	15.67±1.86a-c	13±1.2c-h	27.33±1.33b-f	21.33±0.33d-i
P31	51.07±1.24ab	34.1±3.99c-g	14.67±1.76a-c	11±0.58f-h	18.33±1.76h	15.5±0.76j
P41	39.7±2.93bc	22.3±3.18h	18±2.31a-c	11.67±1.2e-h	24.33±1.45c-h	24±3.06c-g
P45	49.5±0.21a-c	45±2.75ab	13.67±1.67bc	15±1.2a-h	18.67±0.67gh	16.33±0.88ij
P46	43.23±0.57a-c	37.63±1.13a-g	17.33±1.18a-c	15.67±0.33a-h	24.33±2.67c-h	18.33±0.67g-j
P49	43.6±2.8a-c	36.57±7.66a-g	21.33±2.96ab	11.67±0.88e-h	19±4.36gh	19.67±1.86f-j
P50	48.33±1.92a-c	42.8±1.3a-d	14.67±2.4a-c	14±1.73b-h	22.33±1.2e-h	21.67±1.86d-i
P52	46.2±4.98a-c	31.8±2.45e-g	17.67±1.06a-c	15.67±2.19a-h	30±1.2a-d	29.67±0.33b
P61	39.73±4.83bc	37.67±2.87a-g	20.67±0.33ab	12.33±1.33d-h	21.67±1.83e-h	17±0.2if
P63	44.6±2.36a-c	41.6±1.96a-e	14.67±1.76a-c	12±0.58d-h	20±0.58gh	19.83±1.64f-j
P64	48.3±3.47a-c	43.73±2.48a-d	14.67±0.67a-c	11.67±2.4e-h	24.83±2.46b-h	18.67±1.76g-j
P68	54.53±2.07a	35.57±2.52b-g	17.33±1.45a-c	12±1.73d-h	27±1.15b-f	25±0.58b-f
P69	42.1±1bc	41.07±0.07a-f	14±0.1bc	10±1gh	23.67±1.2d-h	23.5±1.5c-h
P71	50.13±0.09a-c	35.2±2.31b-g	15.33±1.2a-c	11.67±0.67e-h	21.67±2.85e-h	21.33±0.67d-i
P76	46.97±1.6a-c	40.9±1.46a-f	19.33±2.81a-c	15.33±3.76a-h	28.17±0.83b-e	24.5±1.8b-f
P82	41.67±3.3bc	41.77±2.35a-e	22.67±1.76a	20.67±2.73a	24.67±0.67b-h	23±2.52c-h
P114	42.47±2.5bc	42.9±1.48a-d	19.67±2.37a-c	12.67±1.67d-h	31±3.51a-c	28±1.53bc
P116	54.8±0.5a	47.13±1.72a	12±1.2c	9.67±1.67h	22±0.1e-h	22±1.15d-i
P120	40.3±3.08bc	32.73±2.96d-g	15±1.53a-c	19±2.52a-c	23.67±0.88d-h	23±1.1c-h
P143	38.83±3.77c	33.13±5.22c-g	21.67±3.71ab	16±2.08a-g	24.33±0.33c-h	20.67±0.88e-j

P159	46.93±4.72a-c	30.77±1.47f-h	20±2.65a-c	19.67±2.33ab	27±2.65b-f	28.67±3.18bc
P161	45.13±2.73a-c	28.83±2.53gh	17±1.2a-c	17.67±1.33a-e	22±2e-h	19.33±0.33f-j
P163	47.6±2.74a-c	46.53±1.68a	15.33±1.45a-c	15.33±0.88a-h	31.33±1.2ab	28.67±2.96bc
P164	48.2±1.07a-c	45.5±0.4ab	15.67±0.67a-c	15.33±0.88a-h	29.33±1.45a-d	30±1.1b
P165	50±3.23a-c	43.23±1.89a-d	21±2.52ab	18±1.15a-d	25.33±3.48b-g	26.67±3.18b-d
P166	41.63±2.42bc	38.47±1.37a-g	18±2.52a-c	9.67±0.88h	30.17±1.09a-d	26.33±1.45b-e
P171	43.83±2.57a	37.63±1.47a-g	14.33±2.4a-c	15±1a-h	34.67±2.4a	38±2.31a
P173	47.73±1.11a-c	38.73±1.77a-g	21.67±1.67ab	16.67±3.48a-f	21±1.53f-h	18.17±0.93h-j
Mean	45.76±2.43	38.39±2.31	17.25±1.76	14.22±1.55	24.92±1.67	23.17±1.45

Stem diameter is linked to mechanical strength and vascular conductance. Under Zn stress, most genotypes showed reduced stem thickness; however, P50 (4.00 mm), P161 (3.86 mm), and P31 (3.74 mm) retained robust stems, potentially supporting sustained transport and growth. Genotypes such as P82 (2.60 mm) and P163 (2.48 mm) experienced notable thinning (Table 3). Leaf width and length, which impact photosynthesis and transpiration, declined in most genotypes under stress. Yet, P171 (7.23 cm) and P116 (7.12 cm) maintained broader leaf surfaces, while P45 (4.53 cm) and P120 (4.41 cm) experienced over 35% reduction. P159 and P164, interestingly, increased leaf width under Zn stress (+25.3% and +18.6%, respectively), a response possibly linked to enhanced turgor maintenance or mesophyll cell expansion (Table 3).

Table 3. Effects of zinc (Zn) stress on root length, stem diameter, and leaf width in pepper genotypes

Genotypes	Root Length (cm)		Stem Diameter (mm)		Leaf Width (cm)	
	Control	Zn Stress	Control	Zn Stress	Control	Zn Stress
P29	16.67±1.86b-f	13.17±1.59fg	3.4±0.03a-e	3.27±0.07a-h	3.8±0.21a-f	3±0.29a-e
P31	10.33±0.67f	14±1.53e-g	3.15±0.39b-e	2.98±0.1b-h	2.6±0.06e-h	2.4±0.31c-e
P41	25.33±2.03a	22±2.31a-c	3.35±0.06a-e	2.9±0.38c-h	3.23±0.15b-h	3.1±0.1a-e
P45	17.67±1.53b-f	24.67±0.88ab	2.99±0.1de	2.82±0.19d-h	2.4±0.21h	2.37±0.23de
P46	21.67±2.33ab	16.33±1.67c-g	3.96±0.12a	3.24±0.11a-h	2.67±0.17e-h	2.33±0.17e
P49	22±2.31ab	20.33±2.85a-f	3.25±0.34b-e	3.54±0.08a-g	3.37±0.8b-h	2.77±0.65b-e
P50	15.67±2.33b-f	26.67±1.45a	3.7±0.16a-c	4±0.16a	4.13±0.2a-c	3.73±0.15a
P52	19.67±2.19a-d	17.67±2.6b-g	3.17±0.1b-e	3.7±0.13a-e	3.73±0.15a-g	3±0.4a-e
P61	13.67±0.67c-f	16.33±0.67c-g	3.61±0.17a-d	3.76±0.1a-d	2.57±0.07f-h	2.63±0.03b-e
P63	15.43±0.88b-f	16±1.1c-g	2.94±0.02e	2.94±0.09b-h	2.63±0.52e-h	2.83±0.2a-e
P64	11.23±2.33ef	11.67±1.2g	3.15±0.2b-e	2.78±0.1e-h	2.9±0.46d-h	2.77±0.15b-e
P68	20.32±1.45a-c	15.33±2.03c-g	3.78±0.15ab	3.47±0.05a-g	3.67±0.09a-g	2.73±0.18b-e
P69	13±0.1c-f	14.33±0.33d-g	3.51±0.23a-e	3.32±0.07a-h	3±0.1c-h	3.43±0.03ab
P71	17±0.1b-f	14.67±2.4c-g	3.1±0.31c-e	2.96±0.18b-h	3.63±0.73a-h	3.2±0.31a-e
P76	17±3.06b-f	19.67±1.76a-f	3.03±0.12de	2.99±0.26b-h	3.83±0.67a-e	2.53±0.03b-e
P82	16.67±1.86b-f	14±0.58e-g	2.91±0.13e	2.6±0.13gh	2.67±0.33e-h	2.33±0.24e
P114	22.67±2.6ab	18±3.79b-g	3.81±0.3ab	3.81±0.13a-c	2.83±0.17d-h	2.8±0.06b-e
P116	19.33±1.67a-d	21.67±3.71a-d	2.9±0.21e	3.17±0.08a-h	4.33±0.17ab	3.3±0.21a-d
P120	17.33±2.33b-f	19.33±2.19b-f	3.01±0.22de	2.99±0.26b-h	3.9±0.42a-d	2.33±0.27
P143	18±1.2a-f	17.67±1.2b-g	3.42±0.17a-e	2.74±0.34f-h	2.53±0.26gh	2.93±0.3a-e
P159	18.33±2.03a-e	19.33±2.4b-f	3.21±0.06b-e	3.4±0.29a-h	2.53±0.55gh	3.17±0.44a-e
P161	15±1.2b-f	19.67±1.67a-f	3.16±0.05b-e	3.86±0.2ab	2.73±0.13d-h	3.17±0.33a-e
P163	12.33±0.67d-f	15.33±2.6c-g	3.57±0.19a-e	2.48±1.13h	3.33±0.33b-h	2.83±0.33a-e
P164	11±2.65ef	21±2.52a-e	3.33±0.19a-e	3.63±0.12a-f	2.53±0.03gh	3±0.12a-e
P165	18.33±2.33a-e	21.67±2.96a-d	3.39±0.14a-e	3.7±0.05a-e	3±0.25c-h	2.73±0.15b-e
P166	12±2.31d-f	13.33±1.86fg	3.65±0.02a-d	3.3±0.15a-h	3.33±0.17b-h	2.6±0.1b-e
P171	22.67±2.78ab	18±2.52b-g	3.65±0.32a-d	3.7±0.13a-e	4.57±0.47a	3.33±0.44a-c
P173	12.33±0.67d-f	10.83±2.05g	3.2±0.1b-e	2.91±0.04c-h	3.2±0.31b-h	2.77±0.23b-e
Mean	16.88± 1.72	17.60±1.94	3.33±0.16	3.25±0.18	3.20±0.29	2.86±0.23

Fresh and dry biomass are robust indicators of plant vigor under stress. P161 (8.82 g), P116 (8.68

g), and P159 (8.63 g) accumulated the highest fresh biomass, whereas P49 (4.65 g) and P173 (4.3 g) ranked lowest. Dry weight measurements mirrored this trend: P165 (1.23 g), P114 (1.2 g), and P171 (1.1 g) retained significantly higher dry biomass compared to P49 (0.67 g) and P173 (0.59 g) under Zn stress ($p < 0.05$). Of particular note, P31 exhibited increases in both fresh (+37.4%) and dry weight (+35.2%) under Zn stress, a rare and valuable trait that may suggest inherent tolerance or priming. Conversely, P173 and P49 experienced more than 45% decline in biomass, reflecting high sensitivity (Table 4).

Table 4. Effects of zinc (Zn) stress on leaf length, fresh weight, and dry weight in pepper genotypes

Genotypes	Leaf Length (cm)		Fresh Weight (g)		Dry Weight (g)	
	Control	Zn Stress	Control	Zn Stress	Control	Zn Stress
P29	8.5±0a-e	6.57±0.35a-d	11.88±1.03a	7.75±0.5ab	1.3±0.02b	0.93±0.01b
P31	6.33±0.33e-j	5.23±0.23b-d	5.46±0.32d	7.5±1.63a-c	0.71±0.01c	0.96±0.02b
P41	7.33±0.83a-j	6.43±0.07a-d	7.85±1.51b-d	7.5±1.72a-c	1.09±0.02b	0.98±0.01b
P45	6.27±1.01e-j	5.67±0.93a-d	6.34±1.08b-d	4.95±0.46bc	0.78±0.01c	0.57±0.02c
P46	5.83±0.33f-j	6.4±0.6a-d	10.07±1.43ab	6.13±0.02a-c	0.81±0.01c	0.48±0.01c
P49	7.1±1.51b-j	5.83±0.93a-d	7.88±0.91b-d	4.65±0.64bc	1.14±0.02b	0.67±0.01c
P50	8±0.58a-g	7.27±0.7a	6.91±0.98b-d	7.35±0.86a-c	1.05±0.02b	1.04±0.01a
P52	7.67±0.44a-i	6.77±0.62a-c	8.62±0.29a-d	6.44±0.56a-c	1.16±0.02b	0.84±0.02b
P61	5.83±0.33f-j	6.33±0.33a-d	9.98±1.92ab	7.34±0.47a-c	0.76±0.01c	0.68±0.01bc
P63	6±0.76e-j	5.03±0.49cd	5.7±0.76cd	7.29±0.09a-c	0.68±0.01c	0.77±0.02b
P64	5.83±0.73f-j	5.57±0.23a-d	7.92±1.23b-d	4.9±0.59bc	0.96±0.02bc	0.67±0.01bc
P68	9±1.15a-d	5.93±0.47a-d	8.63±0.28a-d	6.05±0.55a-c	1.02±0.02bc	0.8±0.01b
P69	5.33±0.17h-j	6.33±0.17a-d	8.16±0.44a-d	6.55±0.35a-c	0.69±0.02c	0.63±0.01bc
P71	7±1.26b-j	5.67±0.44a-d	5±1.46d	4.45±0.49bc	0.8±0.03c	0.6±0.02c
P76	8.1±1.05a-g	5.53±0.53a-d	8.57±1.31a-d	6.17±1.46a-c	0.82±0.01c	0.81±0.01b
P82	5.17±0.17ij	4.67±0.33d	7.37±0.28b-d	5.9±1.08a-c	1.02±0.02bc	0.88±0.01b
P114	5±0.76j	6.07±0.12a-d	10.23±1.15ab	8.45±1.16a	1.6±0.02a	1.2±0.01a
P116	8.33±0.67a-f	6.33±0.88a-d	7.8±1.58b-d	8.68±0.32a	0.59±0.01c	0.7±0.06b
P120	9.1±0.46a-c	5.67±0.33a-d	7.14±0.74b-d	7.48±2.41a-c	0.91±0.01bc	0.9±0.01b
P143	4.87±0.24j	6.4±0.7a-d	6.66±0.26b-d	6.83±1.05a-c	0.85±0.02bc	0.86±0.01b
P159	5.6±1.2g-j	7±0.58ab	6.72±1.68b-d	8.63±1.56a	0.88±0.02bc	0.96±0.02b
P161	7±0.1b-j	6±0.1a-d	6.76±0.14b-d	8.82±0.96a	0.83±0.02bc	0.85±0.02b
P163	6.5±1.32d-j	5.43±0.3a-d	8.08±0.8a-d	7.04±0.82a-c	1±0.01bc	0.93±0.01b
P164	6.67±0.22b-j	5.67±0.6a-d	6.68±1.73b-d	8.74±0.28a	0.92±0.01bc	1.1±0.01a
P165	7.83±0.58a-h	7.23±0.73a	7.04±1.63b-d	8.62±0.8a	1.1±0.01b	1.23±0.03a
P166	9.17±0.73ab	5.67±0.33	8.09±1.16a-d	5.04±0.77bc	1.1±0.01b	0.71±0.01bc
P171	9.73±0.5a	7.17±0.73a	9.57±1.54a-c	8.78±0.87a	1.2±0.02b	1.1±0.02a
P173	6.57±0.54c-j	5.67±0.83a-d	8.25±0.09a-d	4.3±0.36c	1±0.02b	0.59±0.01c
Mean	6.99±0.64	6.06±0.49	7.83±0.99	6.87±0.82	0.96±0.02	0.84±0.02

Percentage-based comparisons emphasize relative tolerance. Root length increased notably in P164 (+90.9%) and P50 (+70.2%), while P46 and P68 had >24% reductions. P159 (+25.3%) and P164 (+18.6%) increased leaf width, while P45 and P120 exhibited 35–40% reductions. Biomass gain in P31 contrasted sharply with losses in P49 and P173 (Fig. 1). These trends reinforce the polygenic and multidimensional nature of Zn stress tolerance.

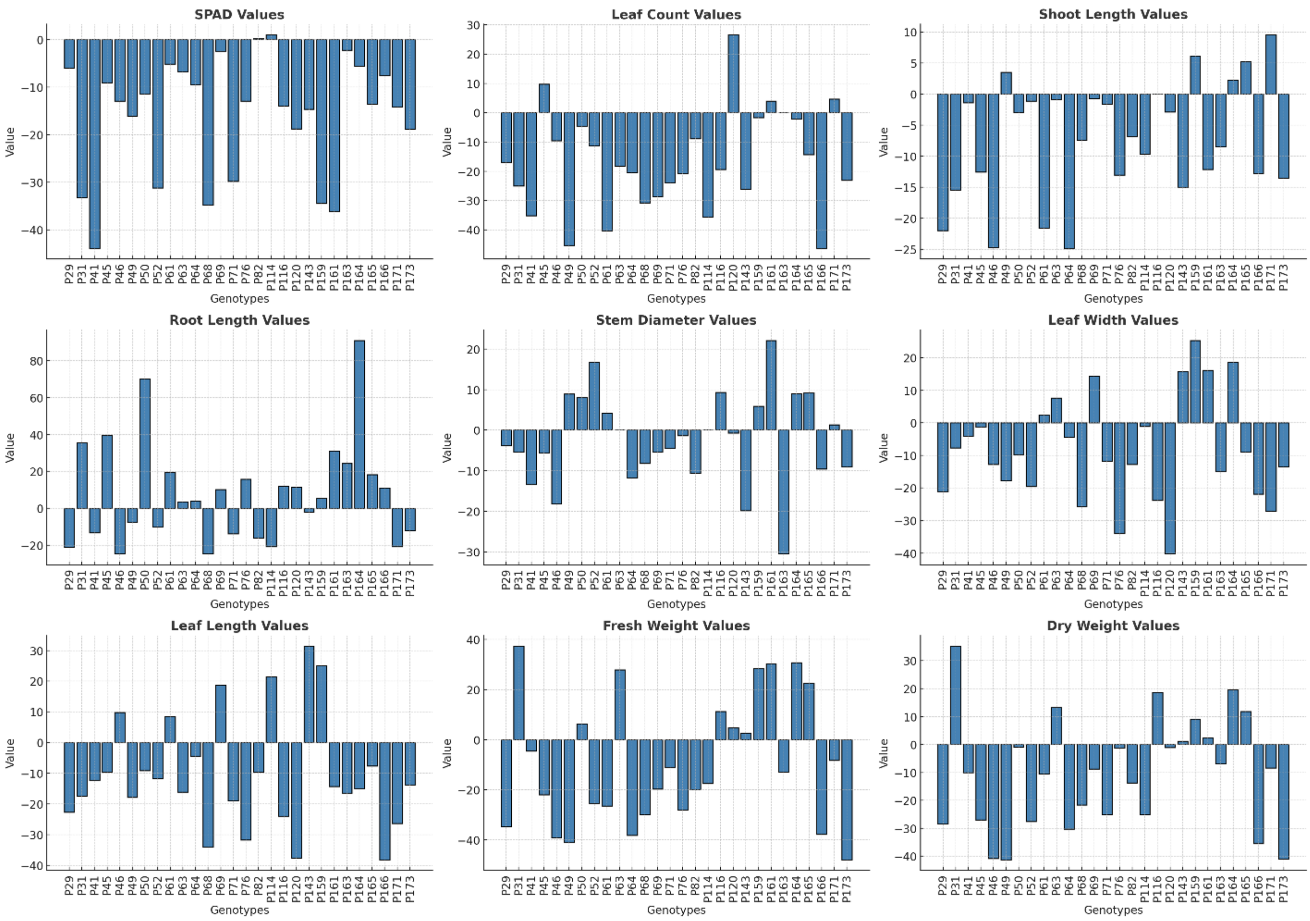

Fig. 1. Percentage change in SPAD values, leaf count, shoot length, root length, stem diameter, leaf width, leaf length, fresh weight and dry weight of pepper genotypes under zinc (Zn) stress compared to control conditions.

PCoA analysis grouped genotypes according to their morphophysiological response patterns. Zn-tolerant genotypes (P164, P114, P159, and P46) formed a distinct cluster characterized by biomass retention, root elongation, and moderate chlorophyll stability. Sensitive genotypes (P29, P76, P166, and P173) clustered separately due to uniform reductions across traits. Correlation analysis confirmed that fresh and dry weight were strongly related (r = 0.90), indicating water content as a major factor in biomass stability. Root length was moderately correlated with dry weight (r = 0.55), highlighting the importance of root system plasticity under stress. Leaf width also showed moderate correlation with fresh weight (r = 0.46), emphasizing its role in photosynthetic capacity. SPAD, however, had a weak negative correlation with biomass (r = -0.27), underscoring that visual greenness is not always indicative of growth performance (Fig. 2).

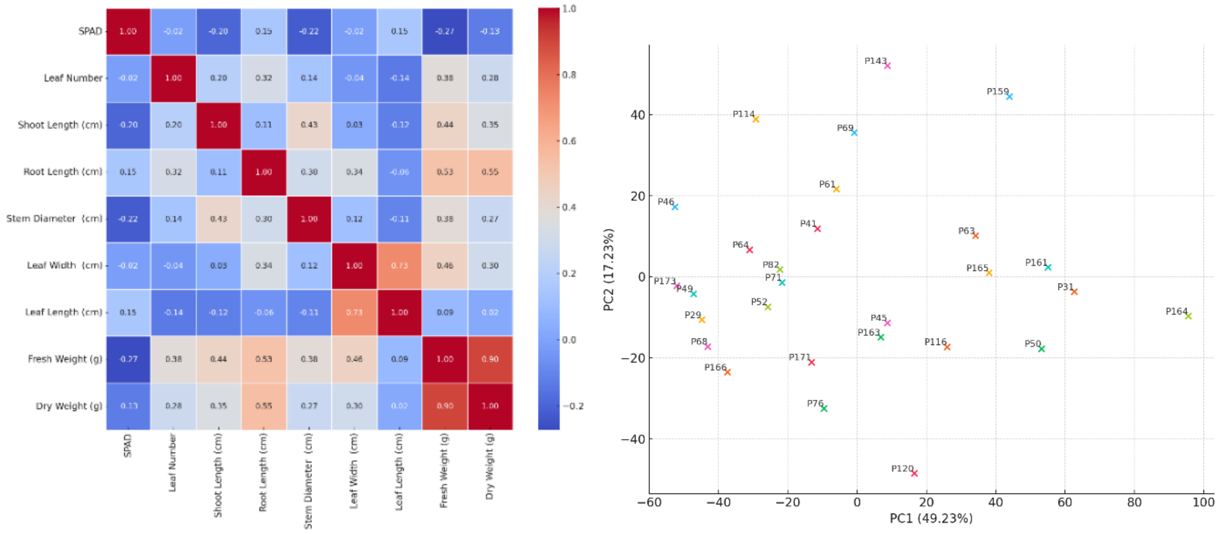

Fig. 2. Correlation matrix of measured parameters under Zn stress and PCoA Biplot of Pepper Genotypes Under Zinc Stress

This study investigated the morphological and physiological responses of 28 C. annuum genotypes to zinc (Zn) stress in a hydroponic system. The results revealed significant inter-genotypic variability in chlorophyll content, shoot and root growth, stem thickness, leaf dimensions, and biomass production. Such phenotypic diversity under stress highlights the potential for selecting Zn-tolerant germplasm in pepper breeding programs. Chlorophyll content (SPAD value) is widely used as a proxy for photosynthetic capacity and plant health under abiotic stress. In this study, genotypes such as P116 and P163 maintained high SPAD values under Zn stress, indicating sustained chlorophyll integrity. These results are consistent with previous findings in tomato and wheat, where tolerant cultivars showed minimal chlorophyll degradation under heavy metal exposure (Farooq et al., 2016; Hasanuzzaman et al., 2019). However, unlike some previous studies that reported a strong association between SPAD values and overall tolerance, our findings showed that genotypes with relatively stable SPAD values did not always produce the highest biomass. This discrepancy may be attributed to species-specific responses, experimental conditions (e.g., Zn concentration, exposure time, developmental stage), or genotype × environment interactions that influence the physiological prioritization between chlorophyll maintenance and biomass allocation. Moreover, in contrast to biomass reduction, SPAD values may recover more rapidly due to dynamic pigment regulation. Shoot and root development are vital for nutrient uptake and plant architecture. Genotypes such as P171 and P164 demonstrated robust shoot elongation, while others like P45 and P31 showed pronounced inhibition. Interestingly, the paradoxical increase in shoot length observed in P171 under stress may reflect an escape response or a hormetic growth stimulation, a phenomenon previously reported under sub-toxic Zn exposure in Arabidopsis (Gao et al., 2020). Differences in hormonal signaling, ROS sensitivity, and energy partitioning might explain variation in shoot growth response among species and genotypes. Root system plasticity plays a crucial role in stress adaptation. The increased root length in P50 and P164 suggests a tolerance mechanism, potentially through improved Zn exclusion or adaptive changes in root architecture. Similar responses have been observed in maize under cadmium and Zn stress, where tolerant lines developed deeper or more branched roots (Lux et al., 2011). Conversely, root stunting in P173 and P46 likely reflects toxicity-induced growth inhibition at the cellular level. Stem diameter and leaf morphology were also found to be stress-responsive traits. The maintenance of stem thickness in P50 and P161 under stress may be associated with efficient vascular transport, supporting shoot integrity. Leaf width and length, which influence photosynthesis and transpiration, varied significantly among genotypes. The expansion of leaf width in P159 and P164 under stress may point to a unique compensatory mechanism, possibly mediated by osmotic adjustment.

Biomass accumulation, a direct indicator of plant performance, revealed clear divergence between tolerant and sensitive genotypes. P31 notably increased both fresh and dry weight under stress, making it a strong candidate for further physiological and molecular investigation. The biomass decline in genotypes like P49 and P173 aligns with their poor performance in other morphological traits, reinforcing their sensitivity to Zn toxicity. The principal coordinate analysis (PCoA) clustered tolerant genotypes (e.g., P164, P159, P114) based on multi-trait performance, supporting their consistent responses across several parameters. This clustering approach allows integrative screening beyond single-trait selection and aligns with recent methodologies used in stress physiology studies (Bista et al., 2023).

Conclusion

This study revealed significant morphological and physiological variation among 28 *C. annuum* genotypes in response to zinc (Zn) stress. Key traits such as chlorophyll content, shoot and root length, stem diameter, and biomass showed distinct genotypic patterns under 5 mM $ZnCl_2$ stress. The identification of Zn-tolerant genotypes (e.g., P164, P114) offers promising material for breeding programs aiming to develop cultivars suited for contaminated soils. These findings are especially relevant for sustainable agriculture in regions where Zn contamination poses a threat to crop yield and food safety. Moreover, the high-performing genotypes may be used as donor parents in stress-tolerant rootstock development or phytoremediation strategies.

Conflicts of Interest

The authors declare that they have no conflict of interest.

Author Contributions

All authors contributed equally to the manuscript. The final version of the manuscript was reviewed and approved by all authors.

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