



Evaluation of seed germination responses of diverse brinjal (*Solanum melongena* L.) genotypes under cadmium stress

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ABSTRACT

Cadmium (Cd) is one of the most toxic, highly mobile, and widely distributed heavy metal contaminants found in the environment. The present investigation was conducted to assess the genotypic variation in seed germination and early seedling growth of 30 brinjal genotypes under Cd stress at concentrations of 1.5, 3, and 6 ppm. It was observed that cadmium stress reduces the germination and early growth attributes including root length, shoot length, and seedling length of brinjal in a dose-dependent manner. Mean germination time and speed of germination were also found decrease with increased Cd level. Similarly, seedling dry weight, seedling vigour index I (SVI-I), seedling vigour index II (SVI-II), and metal tolerance index were significantly reduced under Cd stress. Pearson's correlation analysis and principal component analysis (PCA) identified speed of germination, metal tolerance index, and SVI-I as important traits associated with Cd tolerance during the germination and early seedling stages. Hierarchical clustering based on germination and growth-related traits under the highest Cd concentration (6 ppm) identified IVBR-23, IVBL-22, IVBR-21, IVBR-20, IVBL-25, IVBR-24, IVBHR-19, and IVBHL-27 as Cd-tolerant genotypes that may be further used for breeding and development of Cd-tolerant brinjal cultivars suitable for cultivation in Cd-contaminated environments.

Key words: Cadmium stress, germination %, seedling vigour index, metal tolerance index, PCA.

INTRODUCTION

In recent years, heavy metal pollution resulting from various anthropogenic activities, including fossil fuel combustion, sewage irrigation, mining, metal smelting, improper industrial waste disposal, pesticide use, and excessive fertilizer application, has emerged as a major environmental concern, posing a serious threat to safe and sustainable vegetable production (Liu *et al.*, 10). The ability of plants to accumulate essential metals equally enables them to acquire other nonessential metals. As metals cannot be broken down, their concentration beyond optimal levels in plants adversely affects plant growth and development (Bae *et al.*, 3).

Cadmium (Cd) is one of the most toxic, highly mobile and widespread heavy metal contaminants found in the environment. Major phytotoxic symptoms of heavy metal stress include reduced seed germination, leaf chlorosis, disruption of membrane integrity, oxidative stress, enzyme inhibition, hampered nucleic acid synthesis, inhibited root and shoot growth, and nutritional imbalance (Wang *et al.*, 14). Cadmium accumulation in plants is closely related to their tolerance to Cd. Studies have shown that different crop species and

even different genotypes of the same species differ in their ability to accumulate Cd and thus in Cd tolerance. Screening and breeding of crop varieties with strong Cd tolerance is therefore very important for food safety and soil remediation (Wang *et al.*, 15).

Brinjal (*Solanum melongena* L.) is an important vegetable crop grown worldwide, and according to the first advance estimate for the year 2025-26, India produces 12.91 million tonnes of brinjal from 679 million hectares of area (Department of Agriculture and Farmers Welfare, Government of India: <https://agriwelfare.gov.in/>). Brinjal possesses a broad genetic diversity, with numerous wild relatives and related species cultivated globally. Brinjal is a nutritious vegetable rich in fiber and various minerals, making it a valuable addition to a healthy diet. It has been used as an Ayurvedic medicine to treat diabetes, obesity, and hypertension for ages (Gyanagoudar *et al.*, 5). Selection of brinjal genotypes with high Cd tolerance is an important strategy to address challenges arising from Cd contamination in soil. In this direction, the current investigation was conducted to assess the genotypic variation in brinjal germination under Cd stress.

MATERIALS AND METHODS

Freshly harvested and healthy seeds of 30 genotypes of brinjal namely IVBSR-2, IVBR-23,

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IVBL-32, IVBR-24, IVBHR-19, IVBR-20, IVBR-22, IVBHR-21, IVBHL-27, IVBHR-16, IVBHL-22, IVBL-29, IVBHL-26, IVBHR-17, Kashi Sandesh, IVBHL-24, IVBL-33, IVBHR-24, IVBR-25, IVBL-28, IVBHR-18, IVBL-31, IVBR-21, IVBL-30, IVBL-22, IVBHL-25, Kashi Komal, Kashi Prakash, IVBL-25 and IVBHL-23 were collected from Division of Vegetable Improvement, ICAR-Indian Institute of vegetables Research, Varanasi.

A germination test was conducted to evaluate the responses of brinjal genotypes to cadmium stress under controlled laboratory conditions. Cadmium stress was stimulated using Cadmium chloride. Seeds of brinjal genotypes were first sterilized for 5 minutes in 10% Sodium hypochlorite (w/v), and thereafter, 15 seeds of each genotype were placed between two filter papers premoistened with four different solutions, viz. distilled water, Cd- 1.5 ppm, Cd- 3 ppm, and Cd- 6 ppm. Thereafter, filter papers were fitted into four different glass Petri-dishes, and subsequently incubated in a germination chamber (temperature: 28 °C, humidity: 80– 90%) for 10 days. The number of germinated seeds and other observations were recorded at 24-hour intervals for 10 days. The experiment was arranged in a completely randomized design (CRD) with three replications for each treatment.

The germination percentage, mean germination time, speed of germination, seedling vigour indices (SVI) and metal tolerance index (MTI) were calculated as described by Ruan *et al.* (12), Ranal and de Santana (11), Wardle *et al.* (17), Abdul-Baki and Anderson (1) and Zacchini *et al.* (19), respectively. Root length, shoot length and seedling length were measured after 10 days using a centimeter scale. Then, the average root length, shoot length, and seedling length were calculated. For seedling dry weight estimation, seedlings were first placed in a white paper envelope and then placed in a hot-air oven at 72 °C until a constant weight was observed. Final weight was calculated as the average weight of seedlings per replication.

$$\text{Germination percentage} = \frac{\text{Number of germinated seeds}}{\text{Total number of seeds in plate}} \times 100$$

$$\text{Mean germination time} = \frac{\sum_{i=1}^k N_i T_i}{\sum_{i=1}^k N_i 2a}$$

Where, T_i is the time from the start of the experiment to the i^{th} interval, N_i is the number of seeds germinated in the i^{th} time interval (not the accumulated number, but the number corresponding to the i^{th} interval), and k is the total number of time intervals.

$$\text{Speed of germination} = \frac{N_1}{T_1} + \frac{N_2}{T_2} + \frac{N_3}{T_3} + \dots + \frac{N_k}{T_k}$$

Where, $N_1, N_2, N_3, \dots, N_k$ are the number of germinated seeds observed at time (days or hours)

$T_1, T_2, T_3, \dots, T_k$ after sowing (Not accumulated/ cumulative number, but the number of seeds that germinated at the specific time) and k is the total number of time intervals.

Seedling vigour index I = germination (%) x seedling length (cm)

Seedling vigour index II = germination (%) x seedling dry weight (mg)

MTI = (Dry matter of seedling under Cd stress / Dry matter of seedling under control) x 100

ANOVA and Tukey's Honestly Significant Difference (HSD) tests were conducted using GraphPad Prism, version 10.4.2. All statistical calculations were conducted using significance levels of $p \leq 0.05$, $p \leq 0.01$, and $p \leq 0.001$. Pearson's correlation and principal component analysis (PCA) were performed using GRAPES software, and cluster heatmap analysis was conducted using SPSS version 27.

RESULTS AND DISCUSSIONS

Germination tests are a reliable way to study how heavy metals affect plants because seeds are highly sensitive to environmental stress. Earlier studies have demonstrated that cadmium toxicity adversely affects seed germination, early seedling growth and other germination indices (He *et al.*, 6). In this study, we also observed significant differences ($p < 0.001$) in genotypes (G), treatments (T), and G x T for germination percentage, mean germination time, speed of germination, shoot length, root length, and seedling length (Fig. 1, Table 1). Brinjal seed germination, shoot length, root length and seedling length were significantly inhibited under cadmium stress, and this inhibition was observed to be more pronounced with increasing cadmium concentrations. Seed germination % decreased by 5.05%, 7.14%, and 8.58% under Cd1.5, Cd3, and Cd6, respectively. The highest percentage decrease in germination at Cd6 was observed in IVBHR-16 (34.15%), followed by IVBHR-24 (21.95%). Conversely, IVBL-33 and IVBL-25 exhibited increase in germination percentage by 2.44% and 4.76%, respectively. Earlier variable responses to Cd stress have also been reported in rice and wheat (He *et al.*, 6; Lin *et al.*, 8).

Furthermore, the mean germination time of brinjal under CK, Cd1.5, Cd3, and Cd6 conditions was 5.90, 5.87, 5.88, and 5.78 days, respectively. The mean germination time under CK, Cd1.5 and Cd3 treatments was found non-significant whereas Cd6 was found significantly different with CK, Cd1.5 and Cd3. Additionally, the speed of germination under CK, Cd1.5, Cd3 and Cd6 was 2.45, 2.31, 2.26 and 2.28 days, respectively. Overall, it was clear that mean germination time and speed of germination decrease

Table 1: Analysis of variance indicated highly significant differences among genotypes (G), Cd treatments (Cd), and their interaction (G × Cd).

Trait	Genotypes (G)			Treatment (T)			G × T		
	Mean sq	F	P	Mean sq	F	P	Mean sq	F	P
Germination %	398.3	38.42	<0.001	1081	104.30	<0.001	77.87	7.51	<0.001
Mean germination time (Days)	1.94	140.5	<0.001	0.20	14.59	<0.001	0.23	17.14	<0.001
Speed of germination (Days)	0.67	349.4	<0.001	0.66	347.0	<0.001	0.08	42.79	<0.001
Shoot length (cm)	6.82	1666	<0.001	7.98	1951	<0.001	0.89	219.7	<0.001
Root length (cm)	12.17	1334	<0.001	7.01	769.1	<0.001	2.42	265.9	<0.001
Seedling length (cm)	27.47	1182	<0.001	27.94	1202	<0.001	4.10	176.5	<0.001
Seedling dry weight (mg)	19.64	1064	<0.001	6.05	328.2	<0.001	2.74	148.6	<0.001
Seedling vigour index- I	134796	523.8	<0.001	166898	648.5	<0.001	25692	99.83	<0.001
Seedling vigour Index- II	236827	665.7	<0.001	507265	1426	<0.001	35430	99.59	<0.001
Metal tolerance index	3815	656.0	<0.001	675.8	116.2	<0.001	1142	196.4	<0.001

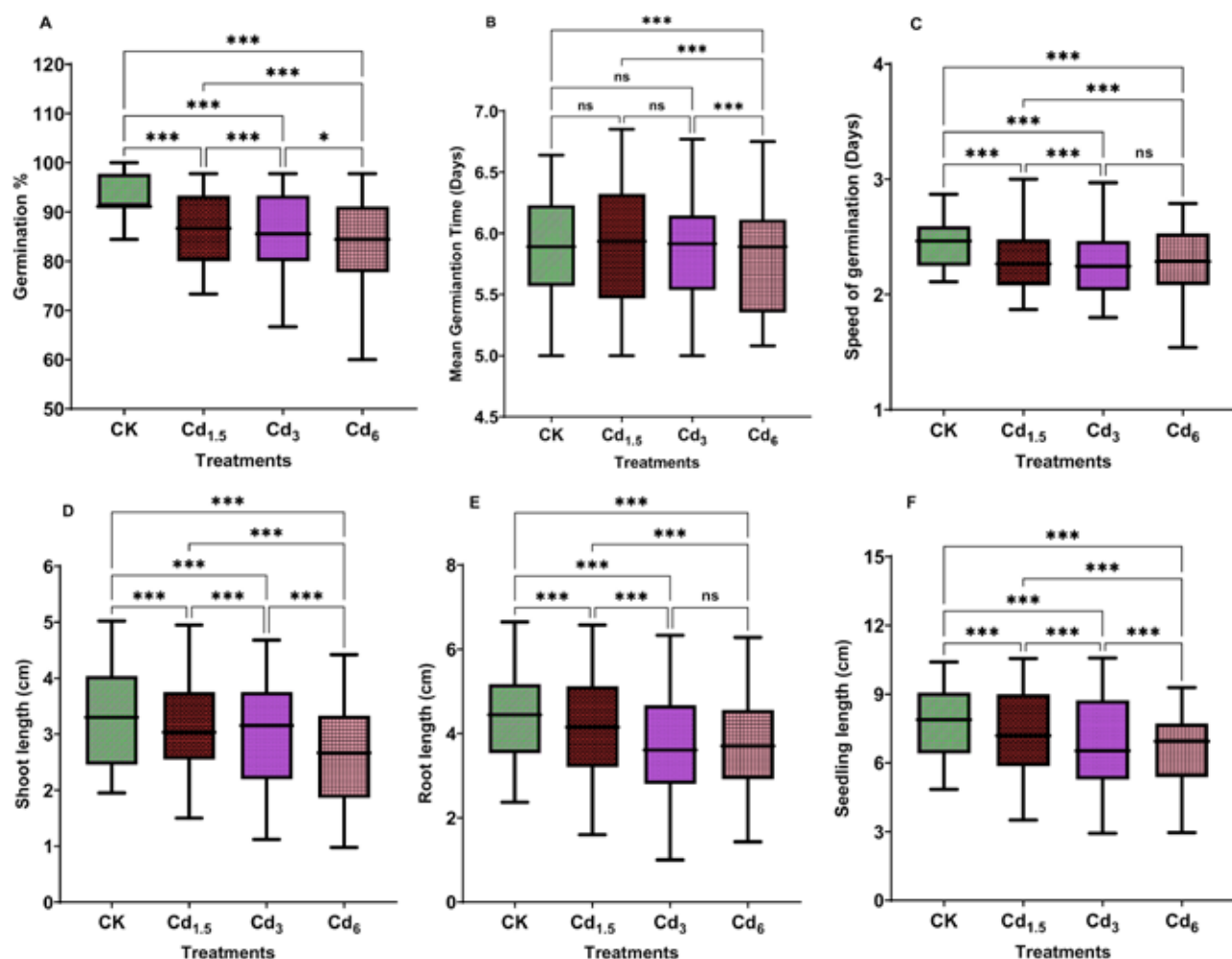


Fig. 1. Box plot presentation of the effect of different levels of Cd on germination % (a), mean germination time (b), speed of germination (c), shoot length (d), root length (e), seedling length (f) of brinjal.

with increased Cd level. Overall, it was evident that both mean germination time and germination speed decreased gradually with increasing Cd concentration. Earlier, the stimulatory effects of low concentrations of heavy metals on seed germination have also been reported in *Ulmus pumila* under cadmium and lead stress (Đukić *et al.*, 4).

Shoot and root length are important factors for any plant in transporting nutrients and water. When exposed to excess Cd, plants may exhibit symptoms such as reduced seed germination, inhibited root elongation and reduced photosynthetic activity (Bae *et al.*, 3). Shoot length decreased by 5.82%, 9.43% and 20.89% under Cd1.5, Cd3 and Cd6, respectively. The highest percentage reduction in shoot length at Cd6 was observed in IVBR-21 (59.03%), followed by Kashi Prakash (57.86%). In contrast, Kashi Sandesh and IVBHL-25 exhibited an increase in shoot length by 82.79% and 15.42%, respectively. Similarly, root length decreased by 6.54%, 13.06%, and 13.72% under Cd1.5, Cd3, and Cd6, respectively. The maximum reduction in root length at Cd6 was observed in IVBR-24 (62.53%), followed by Kashi IVBSR-2 (62.12%). Conversely, IVBHL-26 and Kashi Komal exhibited an increase in root length by 47.17% and 38.80%, respectively. Decreases of 6.23%, 11.47%, and 16.86% were observed in seedling length under Cd1.5, Cd3 and Cd6, respectively. The highest percentage reduction in seedling length at Cd6 was observed in IVBR-23 (59.34%), followed by Kashi IVBR-24 (55.73%). In contrast, IVBHL-26 and Kashi Komal exhibited increase in seedling length by 20.76% and 16.31%, respectively. The reduced germination %, mean germination time, speed of germination, shoot length, root length and seedling length under Cd stress happens due to the fact that under heavy metal stress there has been a sharp decline in hydrolyzing enzymes activities which causes reduced movement of water and reserves towards the embryonic axis (Vijayaragavan *et al.*, 13). It indicates that the sensitivity of growth parameters to Cd stress varied with genotypes.

Shoot dry weight, root length and seedling length are often used as key traits for assessing genotype tolerance to stress due to their sensitivity to Cd toxicity (Wang *et al.*, 16). A significant ($p < 0.001$) difference in genotypes (G), treatments (T) and G x T was observed for seedling dry weight, SVI-I, SVI-II and metal tolerance index (Fig. 2, Table 1). Brinjal seedling dry weight, SVI-I, SVI-II and metal tolerance index were found significantly inhibited under cadmium stress and this inhibition was observed more pronounced with increasing cadmium concentrations. Seedling dry weight declined by 6.27%, 7.74%, and 10.50% under

Cd1.5, Cd3, and Cd6, respectively. The highest percentage reduction in seedling dry weight at Cd6 was observed in IVBHR-24 (38.19%), followed by IVBHL-26 (33.33%). In contrast, IVBR-22 and IVBL-33 exhibited increases in seedling dry wt. of 65.17% and 29.36%, respectively.

SVI-I decreased by 11.42%, 14.47%, and 18.72% under Cd1.5, Cd3, and Cd6, respectively. The highest percentage decrease in SVI-I at Cd6 was observed in IVBHR-24 (51.73%), followed by IVBHR-16 (42.02%). In contrast, IVBR-22 and IVBL-33 exhibited increases in SVI-I by 42.63% and 32.42%, respectively. Similarly, SVI-II was reduced by 11.29%, 17.93%, and 24.62% under Cd1.5, Cd3, and Cd6, respectively. The highest percentage reduction in SVI-II was observed in IVBR-23 (61.17%), followed by IVBR-24 (57.89%) under Cd6. In contrast, Kashi Sandesh and IVBHL-26 exhibited increases in SVI-II of 27.75% and 6.09%, respectively, indicating a stimulatory response to cadmium stress in these genotypes. Further, a concentration-dependent downward trend of MTI has been observed in brinjal. IVBR-22 (165.17) showed the highest MTI at Cd6 followed by IVBL-33 (129.21) and K. Sandesh (119.55), whereas IVBR-25 (69.04) showed the lowest MTI followed

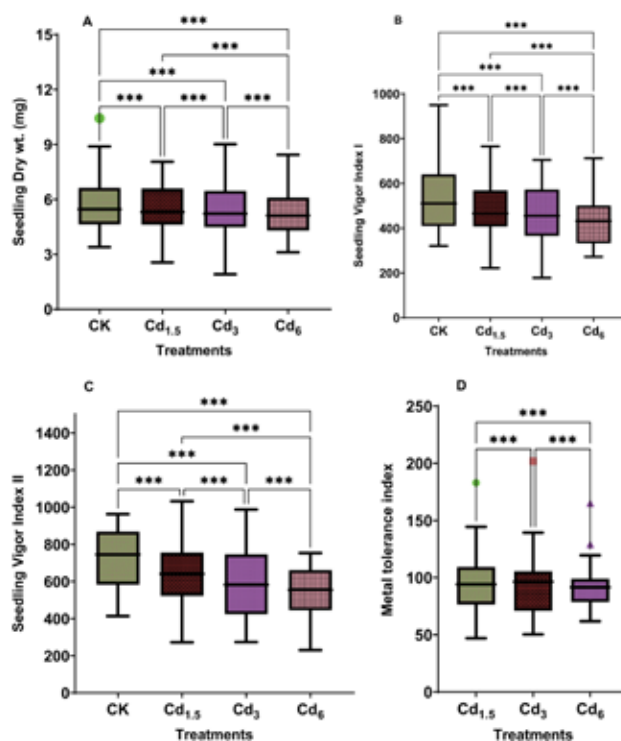


Fig. 2. Box plot presentation of the effect of different levels of Cd on seedling dry weight (a) seedling vigor index I (b), Seedling vigor index II (c), and metal tolerance index (d) of brinjal.

by IVBHL-26 (66.67) and IVBHR-24 (61.78). Earlier studies have also confirmed the relationship between Cd tolerance and root morphological traits (Wang *et al.*, 14). For instance, hot pepper genotypes with low Cd accumulation in fruits exhibited shorter root systems (Huang *et al.*, 7). Similar results have been reported previously in wheat and soybean (Liu *et al.*, 10; Wang *et al.*, 14)

Pearson's correlation analysis was carried out among the studied traits under Cd₀ conditions. MGT was found significantly and negatively correlated with SOG, Root length, Seedling length, Seedling DW, SVI-I and SVI-II. SVI-II was significantly and positively correlated with shoot length, root length, seedling length, seedling DW and SVI-I. Metal tolerance index was significantly and positively correlated with seedling DW and SVI-I (Table 2). Seedling DW was also significantly and positively correlated with root length and seedling length.

PCA analysis revealed that the first three principal components (PCs) have eigenvalues greater than 1 and cumulatively explain 78.3% of total variation. PC1 accounted for 41.5% of the variation with MGT, SOG, shoot length, root length, seedling length, seedling dry weight, SVI- I, SVI-II and MTI being the main factors in PC1 (Fig. 3). PC2 accounted for 21.3% of the variation. MGT was found negatively associated with shoot length, seedling length, seedling dry weight, root length, MTI, SVI-I and SOG. Shoot length, root length, seedling length, seedling DW and SVI-II were found positively associated. Genotypes such as Kashi Sandesh, IVBHR-21, IVBHR-22 and IVBL-33 were positioned towards the positive side of PC1 and in the direction

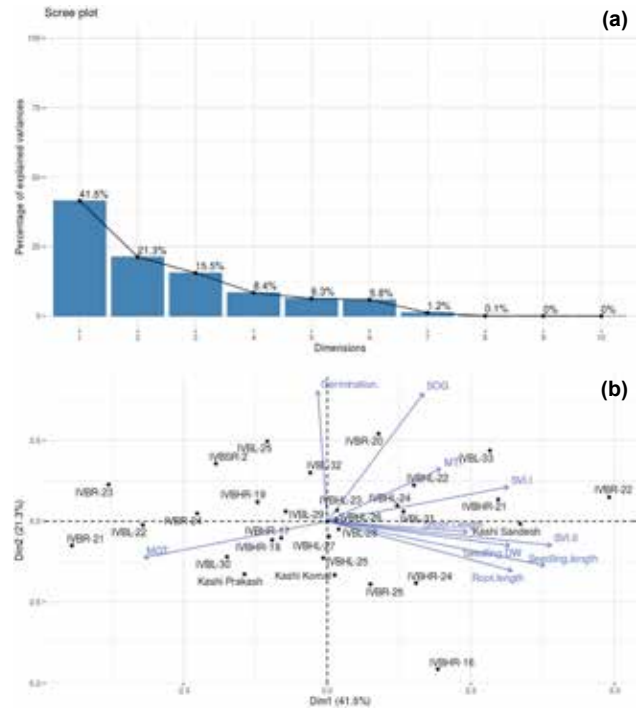


Fig. 3. PCA illustrating the association between different germination parameters of brinjal genotypes under cadmium stress (Cd₀). (a) Scree plot displaying the percentage of variance described by each PC; (b) Biplot representing the interrelationship among germination parameters

of favourable germination/seedling traits, whereas IVBR-21 and other genotypes located in the negative PC1 region were more closely associated with MGT. Correlation and PCA results indicated Cd tolerance

Table 2: Pearson correlation coefficients among different germination parameters of brinjal genotypes under cadmium stress (Cd₆).

Parameter	G %	MGT	SOG	Shoot length	Root length	Seedling length	Seedling DW	SVI-I	SVI-II
G%	1								
MGT	-0.006	1							
SOG	0.828**	-0.558**	1						
Shoot Length	0.048	-0.269	0.18	1					
Root length	-0.238	-0.381*	0.027	0.146	1				
Seedling length	-0.162	-0.438*	0.114	0.632**	0.859**	1			
Seedling DW	-0.305	-0.414*	-0.012	0.16	0.411*	0.405*	1		
SVI-I	0.119	-0.441*	0.358	0.141	0.311	0.316	0.902**	1	
SVI-II	-0.093	-0.556**	0.244	0.691**	0.638**	0.858**	0.471**	0.418*	1
MTI	0.154	-0.343	0.332	0.003	0.229	0.18	0.365*	0.459*	0.142

*Correlation is significant at the 0.05 level.

**Correlation is significant at the 0.01 level.

Table 3: Distribution pattern of 30 brinjal genotypes into three clusters based on euclidean analysis.

Cluster	Genotypes
Tolerant	IVBR-23, IVBL-22, IVBR-21, IVBR-20, IVBL-25, IVBR-24, IVBHR-19, IVBHL-27
Moderate tolerant	IVBHR-21, Kashi Sandesh, IVBHL-22, IVBHL-23, IVBL-33, IVBR-22
Susceptible	IVBL-30, Kashi Prakash, IVBL-29, IVBHR-17, IVBSR-2, IVBL-32, IVBHL-26, IVBHL-24, IVBL-28, IVBL-31, IVBHL-25, Kashi Komal, IVBHR-18, IVBHR-24, IVBR-25, IVBHR-16

in brinjal largely relied on speed of germination, metal tolerance index and seedling vigor index I in the present study (An *et al.*, 2).

Hierarchical cluster analysis using Ward's linkage method revealed substantial phenotypic diversity among the evaluated brinjal genotypes and grouped them into three distinct clusters (Table 3). Previous study in maize it has been also observed that Cd tolerant genotypes have greater root length, than Cd sensitive genotypes (Yang *et al.*, 18). Our study showed that the Cd-tolerant genotypes (IVBR-24, IVBL-22, IVBR-23) have shorter roots, which is a toxic defense response adopted by plants wherein plants limit root growth to reduce metal uptake, protect internal tissues, and conserve energy, although this phenomenon also restricts water and nutrient absorption. Modifications in shoot and root architecture modulate Cd uptake and translocation and are plant strategies for enhanced adaptation to stress (An *et al.*, 2).

Conclusively, our results demonstrated substantial variability in cadmium (Cd) tolerance among the 30 brinjal genotypes based on germination and early seedling growth parameters. Cd stress significantly inhibited seed germination, shoot length, root length and seedling length of brinjal and this inhibition was found more pronounced with increasing cadmium concentrations. The reduction in shoot and root growth under Cd stress may be attributed to impaired water uptake, nutrient imbalance or depletion, and reduced starch mobilization during seed germination. IVBR-23, IVBL-22, IVBR-21, IVBR-20, IVBL-25, IVBR-24, IVBHR-19, and IVBHL-27 were identified as Cd-tolerant genotypes. These genotypes may therefore serve as promising genetic resources for developing Cd-tolerant brinjal cultivars. However, further validation under pot and field conditions is required to confirm their Cd tolerance and assess their agronomic performance under realistic soil Cd stress conditions.

AUTHOR'S CONTRIBUTION

Conceptualization of research (PP); Designing of the experiments (RK, NG); Contribution of experimental materials (SKT, SKM); Execution of experiments and data collection (KK); Analysis

of data and interpretation (RK, KK); Preparation of manuscript and literature review (RK, DRS); Supervision and critical review (PP, AB).

DECLARATION

The authors declare no conflict of interest.

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