Article

Gibberellins Target Shoot-Root Growth, Morpho-Physiological and Molecular Pathways to Induce Cadmium Tolerance in *Vigna radiata* L.

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Citation: Hakla, H.R.; Sharma, S.; Urfan, M.; Yadav, N.S.; Rajput, P.; Kotwal, D.; Abdel Latef, A.A.H.; Pal, S. Gibberellins Target Shoot-Root Growth, Morpho-Physiological and Molecular Pathways to Induce Cadmium Tolerance in *Vigna radiata* L. *Agronomy* **2021**, *11*, 896. <https://doi.org/10.3390/agronomy11050896>

Academic Editor: Charles T. Hunter

Received: 3 April 2021

Accepted: 29 April 2021

Published: 2 May 2021

Abstract: Cadmium (Cd) inhibits plant growth, perturbs nutrient uptake, and affects chloroplast ultrastructure. The role of Cd stress in affecting growth and physiology and ameliorative effects of gibberellins (GAs) in Cd-induced toxicity in mung bean are lesser-known. This study comprehensively investigated Cd stress (CdCl_2 , IC_{50} — $500 \mu\text{M L}^{-1}$) with or without GA_3 on mung bean (*Vigna radiata* L. Var. SML-668). In our methodology, a total of 80 mung bean plants (15 days old of uniform height) were divided into four groups, and each group ($n = 20$) was subjected to four different treatments (Control, CdCl_2 , GA_3 , $\text{CdCl}_2 + \text{GA}_3$) twice during the entire life cycle of mung bean plants (until harvest 85–90 days). Results revealed negative impacts of Cd stress on shoot morphometry (plant height, leaf surface area, stem diameter, shoot fresh weight, number of leaves, number of pods, length, and diameter of pods), root morphometry (root length, root surface area, root dry weight, nodule number and nodule diameter), photosynthetic pigments, and agronomic traits. GA_3 application ameliorated Cd stress by modulating shoot and root growth, improving overall plant metabolism, photosynthetic pigments, and shoot and root morphometry and transcript abundance of *VrPCS1*, *VrIRT1*, *VrIRT2* and *VrCD29*. Thus, we propose GA_3 application for the effective management of Cd-induced phytotoxicity in mung bean plants.

Keywords: cadmium stress; *Vigna radiata*; gibberellic acid; root morphometry; root nodules; pod number

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1. Introduction

Heavy metal (HM) contamination of the biosphere has increased sharply for the last few years and poses major concerns for the environment and human health worldwide. Metals, such as cadmium (Cd), copper (Cu), lead (Pb), and chromium (Cr), could appear in natural and agricultural areas and water bodies. Latest modeling approaches showed mean bioaccumulation factors of heavy metals follow the order $\text{Cd} > \text{Zn} > \text{As} > \text{Cu} > \text{Ni} > \text{Hg} > \text{Cr} > \text{Pb}$ in large number of crops [1]. Among HMs, Cd pollution poses a serious threat to soil quality and human health upon consuming Cd polluted edible crops [2,3]. Hazardously, Cd toxicity is much higher than other organic toxic compounds, due to its greater mobility [4,5]. Cd is hardly degraded in soil, leading to biomagnifications in soil and plants growing in Cd polluted soils. In general, Cd is not a necessary element for plant growth and its excess has a series of harmful effects, including inhibition of plant growth, perturbed nutrient uptake, and chloroplast ultrastructure [4–7]. Cd generates oxidative

stress in plants *via* inducing the production of reactive oxygen species (ROS), such as superoxide anion radicals (O_2^-), hydroxyl radicals, and hydrogen peroxide (H_2O_2) [8,9].

Vigna radiata L. (mung bean) is a widely grown pulse crop in Asia. It is an economical source of protein for direct human consumption [1]. Mung bean is affected by drought [10,11], salinity [12], and other abiotic [13] and biotic stresses [14]. Mung bean plants suffer significantly from HM pollution also in the agricultural soils polluted from effluents of adjoining industrial areas [4–7]. Reduction in plant growth and pod size occurs under HM stress in mung bean [15–17]. Several biotechnological strategies have been adopted towards improving the HM stress management in crop plants [18], such as HM tolerance assisted by microbes [19], transgenics [20,21], and use of phytohormones [22,23]. A strong criticism for transgenic approaches of improving yield and abiotic or biotic stress tolerances of crop plants have impeded the acceptance of transgenic crops by the population world over [24,25]. Eco-friendly approaches (phytohormonal and microbe assisted) of improving HM stress tolerances in crops offers an alternate way without fiddling the genetic make of crop plants and germplasm erosion [26,27]. Literature survey showed scant information about the holistic impact of Cd stress on morphometrical, reproductive, physiological, and agronomical attributes on mung bean plants. Gibberellins (GAs) are important plant hormones implicated in several developmental and physiological processes in plants. GAs role in heavy metal stress management in plants is widely accepted. However, GAs role in reducing Cd-associated stress in mung bean plants is least explored and mostly confined to photosynthesis [28] and seedlings stages [29]. Thus, this study comprehensively investigated the effects of Cd stress on the mung bean plants and the role of gibberellic acid (GA_3 — a type of gibberellins) in mitigating Cd-induced stress markers. Keeping the current understanding of Cd stress in mung bean plants, this study was carried out to observe the impact of Cd stress on growth, as well as biochemical parameters of plants of *V. radiata* from seedling to fruiting stage in the presence of 500 $\mu M/L$ GA_3 concentration and to provide a theoretical basis for the risk assessment of heavy metal pollution and the maintenance of sustainable agricultural production.

2. Materials and Methods

Experiment 1: Growth of Young Plants in Hydroponics

Certified seeds of *Vigna radiata* L. (var. SML-668) were procured from the Agriculture station, Jammu, India. To determine the impact of Cd stress on establishing root and shoot system in young plants, uniform seedlings raised for five days by paper roll method on plain water (control), were subjected to four different treatment regimes viz. Control, $CdCl_2$, GA_3 and $CdCl_2+GA_3$ for 10 days. Young plants were harvested separately into shoot, root, and emerging leaves to measure root and shoot length, and number of emerging leaves and cotyledonary leaves.

Experiment 2: Growth of Plants in Soil

Experiments were conducted in the poly house ($32.72^\circ N$ $74.85^\circ E$) of the Department of Botany, University of Jammu, India. Certified seeds of *Vigna radiata* L. (var. SML-668) were sown in the field beds, at a uniform distance during September 2018 and 2019. At 10 days after sowing (DAS), uniform healthy seedlings were selected and transplanted to pots (26 cm height and 25 cm diameter) filled with 5 kg garden soil mixed with farmyard manure in the ratio of 3:1. A total of 80 mung bean plants (15 days old of uniform height) were divided into four groups, with each group ($n = 20$) subjected to four different treatments (Control, $CdCl_2$, GA_3 , $CdCl_2+GA_3$), for the next 45 days with and without treatment of GA_3 (500 $\mu M/L$) by soil drench method. Water was applied through surface irrigation at the three days interval based on the calculated evapotranspiration demand following the FAO-Penman Monteith model as described elsewhere [30]. Plants were then thoroughly investigated for changes in morphometrical, physiological parameters, and molecular pathways. Prior to $CdCl_2$ application, IC_{50} was calculated for mung bean seedlings grown in Petri dishes over five days.

2.1. Shoot and Root Morphometry and Biomass

The number of cotyledonary and emerging leaves, seedling shoot length (SSL), seedling shoot biomass (SSB), seedling root biomass (SRB), and total root length (TRL), lateral root number (LRN), and TRL/R was measured for young plants grown in hydroponics system using paper roll method. Young root images were analyzed by freely available Image J software Version 1.52v.

For mature plants grown in soil, shoot height (cm), stem thickness (cm), leaf number (N), and leaf surface area (cm²) were measured on a weekly basis. Fresh and dry weights of leaves, stem, and roots were measured at the time of harvest [31]. Root length, root surface area, nodule number, and nodule diameter was measured at the harvesting stage. Root growth was imaged using a high-resolution camera (Nikon B500 Camera, New Delhi, India).

2.2. Histological Localization and Estimation of Cd Accumulation by Atomic Absorption Spectrometry

Histological localization of Cd²⁺ ions was performed with 0.5 M oxalic acid and visualized as white crystalline precipitates. Thin transverse sections of the stem were dipped for 10 min in a solution of 0.5 M Oxalic acid at room temperature. The sections were imaged using a high-resolution camera (Nikon B500 Camera, New Delhi, India). For the measurement of Cd content in mature plants, atomic absorption spectrometry (AAS, Shimadzu, Tokyo, Japan) was used [32].

2.3. Biochemical Estimations and Stress Indices

About 500 mg of fresh leaves were homogenized with ethanol (95%) (5 mL) to make a slurry, samples were centrifuged at 13,000 rpm for 10 min, and estimation of Chla, Chlb, and carotenoids (CAR) [33], sucrose content (a major photosynthate) [34] were carried. Protein estimation, ascorbic acid (ASA), and total phenols (TP) were performed as described [35]. Nodule leg hemoglobin content and Urease enzyme (EC 3.5.1.5) activity was measured by the Berthelot method [36] in the root nodules. Estimation of stress indices such as proline (PL) and malondialdehyde (MDA) was performed as described in Choudhary et al. [37].

2.4. Agronomic Traits

Economically important traits of mung bean plants such as pod diameter, pod length, pod fresh weight, pod dry weight, seed number/ten pods, 100 seeds weight, seeds weight/pod, seed SA, seed diameter, seed protein, and seed sugar content were measured at the harvesting stage using appropriate standard procedures as described elsewhere [38]. Seed attributes particularly, width, length, thickness, surface area, Ew, Ev, geometric mean (Dg), seed shape (Φ %), volume of the grain (V), percent seed germination (%G), and seed protein content (SPr) and seed total phenol content (STPC), were measured.

2.5. Quantitative PCR Analysis

Total RNA was extracted from frozen root samples using TriZol (ThermoFisher, Waltham, MA, USA) with DNase treatment according to the manufacturer's instruction. One microgram of RNA was reverse transcribed using cDNA Synthesis Kit (ThermoFisher, Waltham, MA, USA) with oligodT as primers. Quantitative polymerase chain reaction (qPCR) analysis was conducted on QuantStudio™ 3 Real-Time PCR System (Applied Biosystems, Foster City, CA, USA), and the amplification curve was checked with software provided. The reaction included a SYBR green PCR master mix (ThermoFisher, Waltham, MA, USA) with 4-folds diluted cDNA in the total volume of 20 μ L [35]. A comparative delta Ct ($2^{-\Delta Ct}$) method was used to quantify the relative expression of *Vigna radiata* phytochelatinsynthase 1 (*VrPCS1*), VrIron transporter 1 (*VrIRT1*), VrIron transporter 2 (*VrIRT2*) and Vr cadmium response (*VrCDR29*) with actin as endogenous control. Primer sequences are provided in [39].

2.6. Pearson Correlation Analyses

A pair-wise correlation between Cd stress with or without GA₃ on plant fitness and agronomic attributes was carried out. Pearson's correlation coefficient (r) was calculated using IBM SPSS 20.0 for ten traits (shoot height, stem thickness, leaf number, leaf surface area, root length) of plant fitness and agronomic importance (seed number, seed weight, seed weight/pod and seed surface area). A linear correlation coefficient (r) was calculated to understand the interaction of two factors at a time at $p < 0.05$ and $p < 0.01$ with a number of biological replicates ($n = 20$) for the following combination of treatments CN versus Cd, Cd versus Cd+GA₃ and CN versus Cd+GA₃. The r values fall in the range of +1 to −1, with positive values indicating a positive linear relationship among two treatments and −ve value indicating the negative relationship among treatments.

2.7. Statistical Analysis

Experiments were arranged in a randomized factorial design (2018 and 2019). Data obtained were analyzed by ANOVA and all means were separated at the $p < 0.05$ level using the Duncan test. All calculations and data analyses were performed using the IBM SPSS 20.0 for Windows software package, and the values were expressed by means and standard error (SE).

3. Results

3.1. Growth of Young Shoot-Root System under Cd Stress and after GA₃ Application

The establishment of the young root system, cotyledonary leaves, and emergence of young leaves were affected under Cd stress. The development of cotyledonary leaves is a prerequisite for establishing a healthy young plant. Cd stressed plants showed 13% and 74% reductions in establishment of cotyledonary leaves and the emergence of young leaves compared to control plants (Figure 1a). GA₃ applied to Cd stressed plants showed improvement in establishment and emergence of cotyledonary leaves and young leaves by 15% and 17%, respectively, compared to Cd alone treated plants. GA₃ alone showed no significant reduction in the emergence of young leaves over control (Figure 1a). Seedling shoot length (SSL) reduced by 13% in Cd stressed plants over control. While GA₃ could improve SSL by 20% in Cd stressed plants compared to only Cd-treated plants. About 17% increase in SSL was noted in GA₃ alone treated plants compared to control (Figure 1b). Seedling shoot biomass (SSB) and seedling root biomass (SRB) were reduced by 15% and 29% in Cd stressed plants over control. GA₃ improved SSB and SRB by 22% and 21%, respectively, in Cd stressed plants compared to only Cd-treated plants. No significant change in SSB and SRB occurred in GA₃ alone treated plants over control plants (Figure 1c).

For root growth, Cd stressed plants showed significant impact on establishing the young root system, such that 41%, 26%, and 54% reductions in total root length (TRL), lateral roots number (LRN), and total root length/ roots (TL/R) were recorded in Cd stressed plants over control. GA₃ in Cd stressed plants showed improvements in TRL and TL/R over Cd alone treated plants. GA₃ alone brought reductions in TRL, LRN, and TL/R by 16%, 22%, and 17% compared to control plants (Figure 1d,e).

3.2. Shoot Morphometry under Cd Stress and after GA₃ Application in Pot Experiments

A consistent decrease in stem height and stem diameter was noted in Cd-treated plants (Figure 2a,b), with maximum reductions at 80 DAS. GA₃ steadily improved stem height and stem diameter in Cd-treated plants, with a maximum increase recorded at 70 DAS over Cd-treated plants (Figure 2a,b). GA₃ alone improved stem height significantly at 50–80 DAS and while stem diameter in a non significant manner over control plants. A constant decrease in leaf number was noted in Cd-treated plants; with a maximum reduction at 70 and 80 DAS compared to controls (Figure 2c). GA₃ application to Cd-treated plants could ameliorate negative Cd impact on leaf number, with most significant increases at 70 and 80 DAS over Cd alone treated plants. No significant change in leaf number was recorded for.

Figure 1. Effect of GA₃ on the growth of young mung bean plants under cadmium (Cd) stress in hydroponics. Effect of GA₃ with or without cadmium (Cd) stress on the number of cotyledonary and young emerging leaves (a), seedling shoot length (b), seedling shoot biomass (SSB) and seedling root biomass (SRB) (c), length of the root system (d), total root length (TRL), lateral root number (LRN), and total lateral roots (TLR)/R (e) in paper roll method grown under control (CN), cadmium (Cd) stress, Cd+ gibberellins (GA₃), and GA₃ alone conditions hydroponically. Data presented are means $\pm$ standard errors ($n = 10$, biological replicates). Different letters (a, b) indicate significant differences from control in all combinations (Tukey's test, $p \leq 0.05$).

GA₃ alone except at 80 DAS (Figure 2c). At all observation points, leaf surface area (LSA) was reduced in Cd-treated plants, with maximum reduction recorded at 60, 70 and 80 DAS over control plants. GA₃ improved LSA in Cd-treated plants non significantly at over Cd-treated plants at all observation points (Figure 2d). Similarly, increased LSA was recorded in GA₃ alone treated plants over control plants (Figure 2d).

3.3. Effect of GA₃ on Root Surface Area and Root Nodules Attributes under Cd Stress in Pot Experiments

Root length (RL) and root surface area (RSA) play an important role in the absorption of water and uptake of minerals from the soil solution. In the current study, Cd stressed plants showed significant reductions in RL and RSA over control plants. Furthermore, GA₃ applied to Cd stressed plants increased RSA significantly over Cd stressed plants (Figure 2e,f). No significant change in RSA under GA₃ alone was observed compared to control plants (Figure 2e,f).

Figure 2. Effect of GA₃ on the growth parameters of mung bean plants under cadmium (Cd) stress in soil. Effect of GA₃ with or without cadmium (Cd) stress on stem height (cm) (a), stem diameter (cm) (b), leaf number (N) (c), leaf surface area (cm²) (d), root length (cm) (e), root surface area (cm²) (f), nodule number (g), nodule diameter (cm) (h) of mung bean plants grown in soil in pots at 20 days after sowing (DAS), 30 DAS, 40 DAS, 50 DAS, 60 DAS, 70 DAS, and 80 DAS for (a–d) and 30 DAS, 45 DAS, 60 DAS, and 75 DAS for (e–h) grown under control (CN), cadmium (Cd) stress, Cd+ gibberellins (GA₃), and GA₃ alone conditions. Data presented are means $\pm$ standard errors ($n = 10$, biological replicates). Different letters (a, b, c) indicate significant differences from control in all combinations (Tukey's test, $p \leq 0.05$).

Root nodule number (RNN) in Cd stressed plants decreased at all the observation points (30, 45, 60, and 75 DAS) over control. GA₃ improved RNN in Cd-treated plants at 30, 45 and 75 DAS, while declined it at 60 DAS compared to Cd stressed plants (Figure 2g). GA₃ alone enhanced RNN at 30 DAS, while reduced RNN at 45, 60 and 75 DAS over control (Figure 2g). Root nodule diameter (RND) showed a consistent decline in Cd stressed plants, with a maximum reduction (43%) noted at 75 DAS over control (Figure 2h). GA₃ improved RND in Cd stressed plants significantly at 30, 45 and 75 DAS over Cd stressed plants. RND also improved in GA₃ alone treated plants, with a increase observed at 45, 60 and 75 DAS over control plants (Figure 2h).

Urease activity in root nodules decreased under Cd stress at 30, 45, and 60 DAS, while a nonsignificant increase in activity at 75 DAS occurred compared to control. GA₃ could improve urease activity consistently in Cd stressed plants over Cd-treated plants (Table 1). GA₃ alone could improve urease activity at 45 and 60 DAS compared to control. A consistent reduction in legHb content was recorded in nodules of Cd stressed plants at all observation points (30, 60, and 75 DAS), with a maximum decline 57% noted at 60 DAS over control. GA₃ could improve legHb contents in Cd-treated plants at all the observation points, with a maximum increase of 253% recorded for 60 DAS over Cd stressed plants (Table 1). Furthermore, GA₃ alone reduced legHb content at 30 and 40 DAS, while increased it at 60 and 75 DAS compared to control (Table 1).

Table 1. Effect of cadmium (Cd) stress with or without gibberellins (GA₃) on the physiological attributes of *Vigna radiata* L. on chlorophyll a (Chl a), chlorophyll b (Chl b), carotenoids (CAR), total soluble sugars (TSS) in mg g^{−1} fresh weight, malondialdehyde (MDA, μmol g^{−1} fresh weight), proline (PL), total phenol content (TPC), protein (Prot) content in mg g^{−1} fresh weight, urease activity (UA, μg NH₃ g^{−1} 15 min^{−1}) and nodule leg hemoglobin content (Leg Hb, mg g^{−1} fresh weight) in leaf tissues subjected to control (CN), cadmium stress (Cd), gibberellins (GA₃), Cd+GA₃ at 30 days after sowing (DAS), 45 DAS, 60 DAS, and 75 DAS.

		Chla	Chlb	CAR	TSS	MDA	PL	TPC	Prot	Urease Activity	Leg Hb
CN	30DAS	4.98 ± 0.29 a	1.03 ± 0.06 a	1.02 ± 0.06 a	9.2 ± 0.55 a	0.63 ± 0.03 a	2.83 ± 0.14 a	1.65 ± 0.08 a	1.36 ± 0.06 a	7.22 ± 0.36 a	1.26 ± 0.29 a
	45DAS	6.21 ± 0.37 a	1.38 ± 0.08 a	1.96 ± 0.11 a	12.51 ± 0.75	1.23 ± 0.06 a	3.56 ± 0.17 a	2.56 ± 0.12 a	1.59 ± 0.07 a	8.60 ± 0.43 a	2.54 ± 0.08 a
	60DAS	6.77 ± 0.40 a	1.40 ± 0.08 a	2.03 ± 0.12 a	20.02 ± 1.20	1.54 ± 0.07 a	4.65 ± 0.23 a	3.66 ± 0.18 a	2.34 ± 0.11 a	7.02 ± 0.35 a	4.93 ± 0.07 b
	75DAS	6.42 ± 0.38 a	3.41 ± 0.20 a	1.75 ± 0.10 a	20.36 ± 1.22	1.88 ± 0.09 a	6.56 ± 0.32 a	4.56 ± 0.22 a	3.20 ± 0.16 a	4.02 ± 0.20 a	6.09 ± 0.19 b
Cd	30DAS	2.25 ± 0.13 b	2.63 ± 0.15 b	1.62 ± 0.09 b	5.2 ± 0.31	0.85 ± 0.04 b	8.56 ± 0.42 b	1.99 ± 0.09 a	3.58 ± 0.17 b	6.74 ± 0.33 a	0.79 ± 0.03 a
	45DAS	3.55 ± 0.21 b	2.28 ± 0.13 b	2.02 ± 0.12 a	7.26 ± 0.43	1.65 ± 0.08 b	10.56 ± 0.52 b	2.56 ± 0.12 a	2.15 ± 0.10 b	5.47 ± 0.27 b	2.69 ± 0.17 b
	60DAS	3.42 ± 0.20 b	4.58 ± 0.27 b	2.59 ± 0.15 b	9.42 ± 0.56	2.88 ± 0.14 b	17.23 ± 0.86 b	4.56 ± 0.22 b	3.22 ± 0.16 b	4.91 ± 0.24 b	2.14 ± 0.04 b
	75DAS	3.81 ± 0.22 b	3.58 ± 0.21 a	1.75 ± 0.10 a	11.96 ± 0.71	3.01 ± 0.15 b	19.36 ± 0.96 b	5.66 ± 0.28 a	3.06 ± 0.15 a	4.52 ± 0.22 a	2.77 ± 0.29 b
Cd+GA ₃	30DAS	3.94 ± 0.23 c	4.00 ± 0.24 c	1.63 ± 0.09	5.6 ± 0.33 b	0.72 ± 0.03 a	15.23 ± 0.76 d	1.89 ± 0.09 b	1.42 ± 0.07 a	10.01 ± 0.5 b	0.91 ± 0.03 a
	45DAS	3.97 ± 0.23 b	3.41 ± 0.20 c	2.84 ± 0.17 b	8.50 ± 0.51	1.35 ± 0.06 a	19.65 ± 0.98 d	2.44 ± 0.12 a	2.45 ± 0.12 b	9.54 ± 0.47 a	2.68 ± 0.08 b
	60DAS	4.25 ± 0.25 b	3.51 ± 0.21 c	3.17 ± 0.19 c	18.93 ± 1.13	2.14 ± 0.10 c	20.12 ± 1.00 b	3.66 ± 0.18 a	3.77 ± 0.18 c	9.35 ± 0.46 c	6.04 ± 0.58 c
	75DAS	4.26 ± 0.25 d	4.09 ± 0.24 c	2.90 ± 0.17 b	21.67 ± 1.3	2.04 ± 0.10 a	21.36 ± 1.06 b	4.33 ± 0.21 a	2.70 ± 0.13 b	5.12 ± 0.25 c	8.16 ± 0.42 d
GA ₃	30DAS	5.90 ± 0.35 a	2.67 ± 0.16 b	3.39 ± 0.20	8.7 ± 0.52 c	0.84 ± 0.04 b	4.56 ± 0.22 c	2.15 ± 0.10 b	1.78 ± 0.08 a	7.97 ± 0.39 a	1.02 ± 0.04 a
	45DAS	6.44 ± 0.38 a	3.30 ± 0.19 b	2.49 ± 0.14 b	11.55 ± 0.69	1.65 ± 0.08 b	5.60 ± 0.28 c	2.66 ± 0.13 a	2.19 ± 0.10 b	7.52 ± 0.37 a	4.02 ± 0.29 b
	60DAS	7.25 ± 0.43 a	2.62 ± 0.15 c	2.37 ± 0.14 a	26.6 ± 1.59	2.00 ± 0.10 a	12.56 ± 0.62 c	3.36 ± 0.16 a	3.63 ± 0.18 b	7.66 ± 0.38 a	5.42 ± 0.15 c
	75DAS	7.72 ± 0.46 c	5.73 ± 0.34 b	1.88 ± 0.11 b	31.24 ± 1.87	2.15 ± 0.10 a	16.23 ± 0.81 b	4.56 ± 0.22 a	3.56 ± 0.17 a	7.85 ± 0.39 b	4.58 ± 0.16 b

Data presented are means ± standard errors ($n = 10$, biological replicates). Different letters (a, b, c) indicate significant differences from control in all combinations with respective days after sowing (Tukey's test, $p \leq 0.05$).

3.4. Effect of GA₃ on Plant Biomass under Cd Stress

Plant biomass is a good indicator of plant fitness under abiotic stress. In the present study, Cd stressed plants showed 49% reduction in fresh leaf biomass (FLB) and 29% in dry leaf biomass (DLB) compared to control (Figure 3a,b). GA₃ could improve FLB and DLB by 24% and 63% in Cd stressed plants over Cd-treated plants alone (Figure 3a,b). GA₃ alone improved FLB and DLB compared to control plants. Stem fresh (SFB) and stem dry biomass (SDB) showed a reduction by 12% and 11% in Cd stressed plants over control. GA₃ supplementation to Cd stressed plants witnessed increased SFB (8%) and SDB (9%) compared to Cd alone treated plants (Figure 3c,d). GA₃ alone could improve SFB (11%) and SDB (12%) over untreated control plants. Root biomass is a key determinant for the successful survival of a plant under abiotic stresses (Figure 3e,f). Under Cd stress, 14% and 50% reduction in root fresh biomass (RFB) and root dry biomass (RDB) was noted over control. GA₃ could improve both RFB and RDB by 6% and 35% in Cd stressed plants over Cd-treated plants. GA₃ alone reduced RFB and RDB over control plants (Figure 3e,f).

Figure 3. Effect of GA₃ on the biomass of mung bean plants under cadmium (Cd) stress in soil. Effect of GA₃ with or without cadmium (Cd) stress on fresh leaf biomass (a), dry leaf biomass (b), stem fresh biomass (c), stem dry biomass (d), root fresh biomass (e) and root dry biomass (f) of mung bean plants grown in soil in pots at time of harvest. Control (CN), cadmium (Cd) stress, Cd+ gibberellins (GA₃), and GA₃ alone conditions. Data presented are means $\pm$ standard errors ($n = 10$, biological replicates). Different letters (a, b, c) indicate significant differences from control in all combinations (Tukey's test, $p \leq 0.05$).

3.5. Effect of GA₃ on Photosynthetic Pigments and Soluble Sugar under Cd Stress

Photosynthetic pigments suffered damage under Cd stress such that a consistent decrease in Chl_a content at all the observation points (30, 45, 60, and 75 DAS), with maximum reduction (55%) occurred at 30 DAS compared to control (Table 1). GA₃ could improve Chl_a content consistently, with a maximum increase (75%) recorded at 30 DAS over Cd alone treated plants. GA₃ alone improved Chl_a content at all observation points, with

maximum enhancement (20%) noted at 75 DAS compared to control plants (Table 1). Contrary to Chl a , under Cd stress, Chl b content showed a consistent increase at all observation points, with a maximum increase 225% recorded at 60 DAS compared to control plants. Furthermore, GA $_3$ could increase Chl b content throughout the experiment, with maximum improvement (52%) noted at 30 DAS over Cd alone treated plants (Table 1). GA $_3$ alone could improve Chl b content at all the observation points, with a maximum increase (157%) being recorded at 30 DAS compared to control plants. For carotenoids (CARs), under Cd stress, content showed an upward trend, with a maximum increase (59%) noted at 30 DAS over control plants. GA $_3$ improved CAR content maximally (66%) at 75 DAS compared to only Cd-treated plants. GA $_3$ alone could improve CAR content at all the observation points, with a maximum increase (232%) recorded at 30 DAS compared to control plants (Table 1). Total soluble sugars (TSS) amount was significantly reduced at 30, 45, 60, and 75 DAS in Cd stressed plants compared to control. GA $_3$ supplied to Cd stressed plants significantly enhanced TSS by 81% and 100% at 60 and 75 DAS than Cd alone treated plants. GA $_3$ alone could improve TSS significantly at 75 DAS over control plants (Table 1).

3.6. Effect of GA $_3$ on Stress Indices and Antioxidant System in Pot Experiments

Stress indices such as malondialdehyde (MDA, membrane damage indicator) and proline (PL) play a significant role in evaluating the physiological status of a plant under abiotic stress. In the present study, Cd stress was shown to increase MDA content at all the observation points, with the most significant membrane damage (87%) recorded at 60 DAS compared to control plants (Table 1). GA $_3$ could bring a decline in MDA content in Cd stressed plants maximally at 75 DAS over Cd-treated plants. GA $_3$ alone significantly increased MDA (34%) at 45 DAS compared to control plants. Under Cd stress, PL content increased sharply, with a maximum increase (270%) at 60 DAS compared to control plants. GA $_3$ supplementation to Cd stressed plants further improved PL content at all the observation points, with a maximum increase (86%) recorded at 45 DAS over Cd alone treated plants. A consistent increase in PL occurred under GA $_3$ alone, with a maximum increase (170%) noted at 60 DAS over control plants (Table 1).

Among antioxidants, total phenol content (TPC) increased maximally (25%) at 60 DAS in Cd stressed plants compared to control plants (Table 1). GA $_3$ applied to Cd stressed plants showed a decline in TPC content at all the observation points, with maximum reduction (24%) noted at 75 DAS over Cd alone treated plants. In comparison, GA $_3$ alone treated plants showed a maximum increase in TPC (30%) at 30 DAS (Table 1).

3.7. Effect of GA $_3$ on Accumulation, Translocation, and Localization of Cd Ions

Compared to control plants, Cd stressed plants showed 61%, 126%, and 88% higher accumulation of Cd in leaf, shoot, and root tissue (Figure 4a). GA $_3$ application to Cd stressed plants reduced accumulation of Cd by 35%, 34%, and 46% in leaf, shoot, and root tissues compared to Cd alone treated plants (Figure 4a). Bioaccumulation factor (BAF) from soil to root transfer of Cd showed a marked increase by 14.5 fold-over soil concentration of Cd alone (Figure 4b). GA $_3$ application to Cd stressed plants showed a significant reduction of BAF of roots from the soil by 7.8-fold compared to BAF in Cd alone (by 14.5-fold) (Figure 4b). Translocation factor (TF) from shoot-leaf showed no major change in Cd alone, and Cd stressed plants supplemented with GA $_3$ (Figure 4c). About 75% and 65% accumulation of Cd has been noted in pods and seeds of Cd stressed plants compared to control plants grown in soil in pots. GA $_3$ application reduced Cd accumulation by 67% and 89% in pods and seeds compared to Cd alone treated plants. Compared to Cd stressed plants, significant reductions in Cd ions accumulation were recorded in pods and seeds applied with GA $_3$ plus Cd (Figure 4d). For histological localization of Cd $^{2+}$ ions, white crystalline precipitates of Cd oxalate were observed in the transverse sections (T.S.) of the stem of plants under Cd stress. Localization was more pronounced in the cortex areas of Cd stressed plants, while Cd oxalate crystals were few in GA $_3$ applied Cd plants (Figure 4e–h).

Figure 4. Effect of GA₃ on accumulation, translocation, and localization of cadmium (Cd) in mung bean plants. Effect of GA₃ on cadmium (Cd) accumulation (ppm/g dry weight) in leaf, shoot, and root tissue (a), bioaccumulation factor (BAF, Cd accumulation root/soil) (b), translocation factor (shoot/root and leaf/shoot) (c), Cd accumulation (ppm/g dry weight) in pods and seeds (d), and histological localization of Cd ions as Cd-oxalate white crystals in stem tissue transverse sections, Cd alone (f,g) and GA₃ plus Cd (e,h) of mung bean plants grown in soil in pots at time of harvest. Control (CN), cadmium (Cd) stress, Cd+ gibberellins (GA₃), and GA₃ alone conditions. Data presented are means $\pm$ standard errors ($n = 10$, biological replicates) for (a–c). Scale bars: 1000 μ m for (e,f) and 100 μ m for (g,h). Red circles and red arrows represent the localization of Cd oxalate crystals. Different letters (a, b, c) indicate significant differences from control in all combinations (Tukey's test, $p \leq 0.05$).

3.8. Effect of GA₃ on Genes Regulating Cd Uptake and Transport

Expression of genes inducing Cd²⁺ ions sequestration was enhanced under Cd stress compared to the control. Such that 1.7, 2.5, 1.6-fold increase in expression of *VrPCS1*, *VrIRT1*, and *VrIRT2* was noted in Cd stressed seedlings compared to control. GA₃ application further elevated expressions of *VrPCS1*, *VrIRT1* and *VrIRT2* over Cd treatment (Figure 5a–c). GA₃ alone could not modulate expressions of these genes significantly compared to control. Increase in uptake of Cd ions under Cd stressed plants over control was linked with elevated expression of *VrIRT1* and *VrIRT2* over control plants. GA₃ application lowered Cd accumulation in young plants; this reduction in Cd ions accumulation could be attributed to a decline in expression of *VrIRT1* and *VrIRT2* compared to only Cd-treated

seedlings (Figure 5b,c). GA₃ alone also improved *VrIRT1* expression over control though insignificantly compared to control. Elevated expression, 2.2-fold of *VrCDR29* noted in Cd stress alone compared to control. GA₃ application to Cd stress further improved *VrCDR29* expression by 1.7-fold compared to only Cd treatment (Figure 5d).

Figure 5. Effect of GA₃ on the relative gene expression of *Vigna radiata* phytochelatin synthase 1 (*VrPCS1*), (a), *VrIron* transporter 1 (*VrIRT1*) (b), *VrIron* transporter 2 (*VrIRT2*) (c) and *Vr* cadmium response (*VrCDR29*) (d) in young seedlings subjected to Control (CN), cadmium (Cd) stress, Cd+ gibberellins (GA₃), and GA₃ alone conditions in paper roll method. Data presented are means $\pm$ standard errors ($n = 3$, biological replicates). Different letters (a, b, c) indicate significant differences from control in all combinations (Tukey's test, $p \leq 0.05$).

3.9. GA₃ Improves Agronomic Traits Affected by Cd Stress

A visible reduction in pod length and seed size was observed under Cd stress, while GA₃ application could mitigate negative impacts of Cd stress on pod length and seed size (Figure 6). For pod length, Cd-treated plants showed 21% reduction, while GA₃ improved pod length by 11% in Cd stressed plants over Cd alone (Figure 7a). Cd-treated plants showed an 8% reduction in pod diameter compared to control plants. GA₃ could improve pod diameter by 17% in Cd stressed plants (Figure 7b). GA₃ applied alone increased pod diameter by 58% over control plants (Figure 7b). Approximately 34% reduction in pod number/plant was recorded in Cd stressed plants over control plants.

About 25% increase in pod number/plant was recorded for GA₃ applied Cd stressed plants over Cd alone treated plants. No significant change in pod number/plant was recorded for alone GA₃ treated plants over control plants (Figure 7c). About 49% and 52% reduction in pod fresh and pod dry weight was noted in Cd stressed plants compared to control plants. GA₃ in Cd stressed plants showed significant improvement in pod fresh (57%) and pod dry weight (107%) over Cd alone treated plants (Figure 7d,e). Interestingly, a 108% and 237% increase in pod fresh and pod dry weight was recorded for alone GA₃ treated plants compared to control plants (Figure 7d,e).

Figure 6. Effect of GA_3 on pod and seed size attributes under Cd stress. Control (CN, (a,b)), Cd stress (Cd, (c,d)), GA_3 (e,f) and Cd+ GA_3 (g,h) conditions.

For seed, Cd stress was able to influence several important seed attributes viz. seed number/ten pods for normal and aborted seeds, 100 seeds weight, seeds weight/pod, seed width (W), seed length (L), seed thickness (T), seed surface area (SA), mean geometric diameter (D_g), sphericity ($\Phi\%$), seed volume (V) and seed germination percentage (%G). Such that 19%, 40%, and 47% reduction in seed number/ten pods, 100 seeds weight and seed weight/pod was recorded in Cd stressed plants over control plants. However, GA_3 application in Cd stressed plants showed 32%, 35%, and 67% improvement in these seed attributes over alone Cd-treated plants (Figure 7f–h). GA_3 alone improved seed number/ten pods by 9.5% and reduced 100 seeds weight and seed weight/pod by 20% and 10%, respectively, compared to control plants (Figure 7f–h). For seed geometric attributes, about 13%, 15%, 14%, and 16% reduction in W, L, T, and seed surface area (SA) was recorded in Cd stressed plants compared to control plants (Figure 7i, Table 2). GA_3 application to Cd stressed plants improved W, L, T, and SA by 8.47%, 15.29%, 8.23%, and 13.27%, respectively, over Cd alone treated plants. Similarly, 13.77% and 35.89% reduction in D_g and V was recorded in Cd stressed plants over control plants. GA_3 application in Cd stressed plants showed 10.46% and 34.79% improvement in D_g and V over Cd alone treated plants. Contrary, GA_3 application alone reduced D_g by 8.31% and V by 22.91% compared to control plants alone. While no significant change in $\Phi\%$ was recorded in Cd stressed plants, though an insignificant decrease of 4% was recorded for GA_3 applied Cd stressed plants compared to alone Cd-treated plants (Table 2). Seed protein content under Cd stress declined 36% compared to control seeds. Application of GA_3 in Cd stressed plants further declined seed protein content by 20% over Cd-treated plants. Interestingly, seed protein content declined by 35% in GA_3 alone treated plants over control plants (Table 2). Seed sugar content decreased significantly in Cd stressed plants over control (Figure 7j). GA_3 alone or with Cd stress improved seed sugar content significantly (Figure 7j). Seed TPC in Cd stressed plants showed a significant increase (241%) over control plants. GA_3 applied Cd stressed plants showed a 92% reduction in seed TPC over TPC of seeds of Cd alone treated plants. About 43.46% reduction in seed TPC was noted in alone GA treated plants compared to control (Table 2).

Figure 7. Effect of GA₃ with or without cadmium (Cd) stress on pod length (a), pod diameter (b), pod number/plant (c), pod fresh weight/plant (Pod FW, d), pod dry weight (Pod DW, e), normal and aborted seed number/ten pods (f), hundred seeds weight (g), seed weight/pod (h), seed surface area (SA) (i) and seed sugar (j) of mung bean plants grown in soil in pots at time of harvest. Control (CN), cadmium (Cd) stress, Cd+ gibberellins (GA₃), and GA₃ alone conditions. Data presented are means ± standard errors ($n = 10$, biological replicates). Different letters (a, b, c) indicate significant differences from control in all combinations (Tukey's test, $p \leq 0.05$).

Table 2. Effect of cadmium (Cd) stress with or without gibberellins (GA₃) on seed geometric attributes viz. width (W), length (L), thickness (T), Sphericity (S), E_w, E_v, geometric mean (D_g), seed shape (Φ%), volume of the grain (V), gravity (%G), seed protein content (SP_r, mg g^{−1} dry weight) and seed total phenol content (STPC, mg g^{−1} dry weight) of seeds of mung bean plants subjected to control (CN), cadmium stress (Cd), gibberellins (GA₃), Cd+GA₃ at 30 days after sowing (DAS), 45 DAS, 60 DAS, and 75 DAS. Data presented are means ± standard errors (*n* = 10, biological replicates). Different letters (a, b, c) indicate significant differences from control in all combinations (Tukey's test, *p* ≤ 0.05).

Treatment	W	L	T	S	E _w	E _v	D _g	Φ%	V	%G	SP _r	STPC
CN	3.94 ± 0.13 a	4.85 ± 0.20 a	3.95 ± 0.27 a	17.27 a	1.23 a	0.99 a	4.21 ± 0.25 a	86.76 a	39.76	95 a	1.08 a ± 0.01	0.63 ± 0.002
Cd	3.42 ± 0.24 b	4.12 ± 0.66 b	3.40 ± 0.26 b	14.46 b	1.20 a	1.00 a	3.63 ± 0.21 b	87.97 a	25.49	52 b	0.69 b ± 0.002	2.17 ± 0.025
Cd+GA ₃	3.71 ± 0.24 a	4.75 ± 0.48 a	3.68 ± 0.23 a	16.38 a	1.28 b	1.00 a	4.01 ± 0.24 a	84.39 a	34.36	78 c	0.55 b ± 0.001	0.17 ± 0.001
GA ₃	3.60 ± 0.24 a	4.51 ± 0.33 a	3.55 ± 0.17 b	15.65 a	1.25 a	1.01 a	3.86 ± 0.23 a	85.57 a	30.65	97 a	0.70 a ± 0.001	0.36 ± 0.001

3.10. Pearson Correlation Analysis

A significant linear correlation (r) for stem height, stem diameter, and leaf number were noted for Cd stress alone (Table 3). Pearson's correlation coefficient (r) for shoot traits under Cd stress showed linear correlation; however, a significant positive correlation was noted for stem diameter (Table 3). A negative correlation was noted for root length under Cd stress (Table 3). GA₃ applied to Cd stressed plants showed a positive correlation for stem diameter, leaf number, and root length, while a negative correlation noted for stem height and leaf surface area (Table 3). For agronomic traits, seed number, seed weight, seed weight/pod showed a positive correlation with Cd stress, while a negative correlation was found for seed surface area (Table 3). While all agronomic traits except seed number showed a negative correlation between GA₃ applied Cd stressed plants and Cd alone treated plants (Table 3).

Table 3. Pearson correlation coefficient (r) for ten traits of plant fitness (stem height, stem diameter, leaf number, leaf surface area, and root length) and of agronomical attributes (seed number, seed weight, seed weight/pod, seed surface area) for Control versus Cd, Control versus Cd+GA₃ and Control versus GA₃.

Pearson Correlation for Morphometric Traits					
	Stem Height	Stem Diameter	Leaf Number	Leaf Surface Area	Root Length
CN	1	1	1	1	1
Cd	0.364	0.593 *	0.014	0.447	−0.289
Cd+GA ₃	−0.050	0.875	0.582 *	−0.061	0.473
Pearson Correlation for Agronomic Traits					
	Seed Number	Seed Weight	Seed Weight/Pod	Seed Surface Area	
CN	1	1	1	1	
Cd	0.127	0.075	0.295	−0.156	
Cd+GA ₃	0.088	−0.413	−0.400	−0.032	

Number of biological replicates ($n = 13$) across treatments. Asterisk * represents whether the correlation is significant at the 0.05 level (2-tailed).

4. Discussion

Cadmium (Cd) is a nonessential heavy metal known to affect human health and plant growth and metabolism [2,40]. Being highly active Cd, its excess in soil often accounts for perturbed metabolism and growth defects leading to reduced yield in crops of commercial importance [4]. The decrease in shoot and root lengths and fresh and dry biomass, due to heavy-metal stress has been reported in mungbean [8], *Arachis hypogea* [41], and sunflower [42]. The reduction in plant height under Cd stress can be supported with similar observations recorded in *Vigna radiata* (L.) Wilczek cultivars PDM-139 and K-851 subjected to 0.2 mg/L of Cd exposure showed a significant reduction in plant height [43]. However, improved plant height recorded in Cd stressed plants upon application of GA₃ compared to Cd stressed plants could be attributed to the positive impact of GA₃ on inducing plant growth (Figure 1). Our observations are supported by similar findings, wherein application of GA (50 μ M) to *Brassica napus* L. grown in Cd (0, 50, and 100 μ M) hydroponics could increase plant height compared to only Cd hydroponics [44]. Similar to our observations of reduced leaf number and leaf SA in mung bean plants under Cd stress, reduction in leaf number and leaf SA have been reported in *V. radiata* (L.) Wilczek was subjected to increased levels of Cd in the soil medium [45]. Improved leaf SA of Cd stressed plants applied with GA₃ could be linked with the established impact of GAs on the leaf elongation rate (LER) via activating expression of genes of cell elongation, i.e., α -expansins, one β -expansin, and xyloglucan endotransglycosylase [46]. Reductions in both fresh and dry biomasses of Cd stressed plants could be attributed to Cd interference in the normal functioning of enzymes of the Calvin cycle, CO₂ fixation, phosphorus, and carbohydrate metabolism leading to stunted plant growth and inhibition of photosynthesis [19,47].

Plants often respond to soil HM stress by reducing root length and root surface area, to minimize the root area exposition to HM pollution [48]. The decrease in root length, root surface area, and root biomass both in young plants (Experiment 1) and mature plants (Experiment 2). The role of Cd in decreasing nodulation and activity of nitrogen metabolizing enzymes has been established [49]. Higher concentrations of Cd have been observed to induce nodule senescence and decrease in legHb content in leguminous plants [49]. Contrary to earlier reports, Cd stress was observed to increase nodule number/plant over control plants; interestingly, this increase in nodule number/plant was found to be associated with a significant reduction in nodule diameter and nodule surface area. However, the application of GA₃ was able to improve nodule diameter at all observation points with a maximum increase recorded at 75 DAS compared to only Cd stressed plants. Similar to observations reported earlier, Cd stress lowered the nodule legHb content significantly over control plants; however, supplementation of GA₃ to Cd stressed plants showed improvement in nodule legHb content. This reduction in legHb content under Cd stress could be attributed to the production of a higher number of reactive oxygen species with a detrimental impact on nodules [50].

A positive correlation existed between the accumulation of Cd in shoot tissue and its localization. Significantly accumulated Cd in Cd stressed plants followed shoot > root > leaf, while GA₃ application could reduce Cd accumulation and root-shoot and shoot to leaf translocation significantly. Similarly, the accumulation of Cd was higher in pods than seeds, while GA₃ application reduced this accumulation by a significant factor. This significant reduction in Cd accumulation in GA₃ treated plant tissues and its translocations from root to shoot and shoot to leaf could be linked with elevated expressions of genes regulating Cd uptake (*VgCDR*), phytochelatin synthesis (*VgPCS1* and *VgPCS2*), and *VgIRT1* compared to Cd stressed plants.

Reductions in photosynthetic pigments (Chl*a*, Chl*b* and CAR) in Cd stressed plants may be linked to the inhibitory impact of Cd-induced generation of ROS and lipid peroxidation and inhibiting Chl biosynthesis by competing with Mg ions, consequently leading to damage of PS II [51]. Application of GA₃ ameliorated the negative impact of Cd on photosynthetic pigments via reducing the production of ROS and damage to the cell membrane by improving antioxidant activity of Cd stressed plants compared to Cd alone treated plants. Exogenous applications of GA₃ have been shown to improve the contents of photosynthetic pigments by stabilizing and inhibiting the dissociation of the light-harvesting complex from PS II core, under Cu stress in *Helianthus annuus* L. [52].

Cd stressed plants showed an increase in membrane damage as indicated by enhanced levels of MDA throughout the plant's life cycle after the imposition of Cd stress. Application of GA₃ was observed to reduce membrane damage in Cd stressed plants, probably via reducing the production of ROS compared to Cd stressed plants (Table 1). Findings agree with Cd-induced membrane damage and higher production of superoxide radicals and membrane lipid peroxidation products recorded in seedlings of *Vigna aconitifolia* L. [53]. Enhanced levels of PL in Cd stressed plants could be linked with effective HM stress management; moreover, GA₃ induced further increase in PL content in Cd stressed plants can be associated with reducing ROS production. GA₃ induced increase in PL production and its implication in bud outbreak has been recorded in *Prunus avium* (sweet cherry) [54].

Phenolic acids play pivotal roles in abiotic stress management, particularly by neutralizing or scavenging ROS. TPC is a good indicator of total phenolics content in a plant system under abiotic stresses in plants. In the present work, enhanced TPC in Cd stressed plants could be linked with the plant's inbuilt response towards enhanced ROS production and their management. While GA₃ induced a small decrease in TPC of Cd stressed plants could be attributed to the reduction of ROS levels and returning of normal growth conditions compared to Cd stressed plants. Enhanced levels of total phenolics in GA-treated *Vitis vinifera* L. cv. Muscat has been recorded to play important roles in the development of grapevine tissues [55]. Soil HM pollution has been shown to affect the activity of nitrogen metabolism enzymes such as urease and nitrification dynamics in the soil-rhizosphere

zone [56]. At low concentrations (0.5 mg/kg soil), Cd^{2+} cations were able to up-regulate the urease activity; though at higher concentration activity got declined considerably [56]. Similar observations were also recorded for Cd stressed plants and elevated activity of urease activity in GA_3 applied plants.

Cd stress has been observed to reduce agronomic traits of *V. radiata* L. [15], such that plants subjected to 3, 6, 9, and 12 mg/kg soil Cd toxicity showed a reduction in the number of pod/plant, pod length, pod fresh weight, pod dry weight, number of seeds/pod and number of seeds/plant [15]. This investigation also witnessed a reduction in pod size, pod length, pod diameter, pod fresh and dry weights, seed number/pod and plant, seed weight under Cd stress (Figures 6 and 7). Besides, reduced seed surface area, sugar content, protein content, and geometric attributes were also noted under Cd stress. GA_3 supplementation was able to significantly mitigate the negative impact of Cd stress on all the prementioned agronomic attributes.

A Pearson correlation analysis also revealed the negative impact of Cd stress on agronomic and growth parameters of vigna plants. Moreover, GA_3 application improved the negative impact of Cd stress on the growth and agronomic traits considerably. Due to these findings, we propose the application of GA_3 for significantly reducing the Cd stress-associated damages in mung plants.

5. Conclusions

This study demonstrated the negative impact of Cd toxicity on mung bean plants at the morphological, physiological, and agronomical level. GA_3 application was observed to reduce Cd-induced stress through several morphological and physiological adaptations without compromising economic attributes. We propose GA_3 application for the management of Cd-induced stress in mung bean plants.

Author Contributions: Conceptualization: S.P., N.S.Y. and A.A.H.A.L.; methodology: H.R.H., M.U., S.S., P.R. and D.K.; software: S.P.; validation: S.P., H.R.H., M.U., S.S., P.R. and D.K.; formal analysis: H.R.H., M.U., S.S. and D.K.; investigation: H.R.H., M.U., S.S. and D.K.; writing—original draft preparation: S.P., N.S.Y. and A.A.H.A.L.; writing—review and editing: S.P., N.S.Y. and A.A.H.A.L.; visualization: S.P.; supervision: S.P. and A.A.H.A.L.; project administration: S.P.; funding acquisition: S.P. and A.A.H.A.L. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by UGC-SAP-DRS, and Taif University Researchers Supporting Project number (TURSP-2020/72), Taif University, Taif, Saudi Arabia.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: Authors would like to thank Department of Botany, University of Jammu for extending laboratory facilities and technical support. The research work was supported by CSIR-Junior Research Fellowship, Government of India to Urfan Mohammad.

Conflicts of Interest: The authors declare no conflict of interest.

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