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Cadmium-induced alterations in sulphur containing defense compounds (SODC), protease activity, and ultra-structure of mitochondrion and chloroplast in *Brassica juncea* L. genotypes differing in Cd-sensitivity

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Abstract

Cadmium contamination in agricultural soils is an ongoing process and its impact on quality and quantity of cash crops is irreparable. It interferes with the metabolism of most versatile element of organisms (sulphur) after being incorporation in the food chain at all levels. Under these circumstances, the selection of tolerant/resistant genotypes of economically important crops with higher biomass accumulation may be of great help. We isolated Cd-tolerant and Cd-sensitive cultivars/genotypes of Brassica juncea by testing its ten genotypes at seedling stage using the alterations in sulfur metabolic pathways as an indicator of Cd-tolerance.

The series of experiments that we conducted included the assessment of accumulation and compartmentalization of Cd in root, shoot, and impact of Cd on content of soluble protein, free amino acids, sulfur-containing defense compounds like non-protein thiol and phytochelatins. Modulation in activities of proteases and ATP-sulphurylase was also monitored along with modifications in ultra-structure of chloroplast and mitochondria under Cd-stress. Ten days old seedlings of *Brassica juncea* L. genotypes were treated with several levels of $CdCl_2$ (0.0-2 mM).

Among plant organs, root accumulated maximum Cd content followed by shoot, respectively with maximum levels in cv. Among the ten genotypes studied under Cd-stress, cv. Pusa Jai Kisan showed least damage to chloroplast, mitochondrion and soluble protein content but maximum

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increase in accumulation of non-protein thiols and phytochelatins were observed. In the same genotype, ATP-sulphurylase activity was found maximum along with least protease activity and levels of free amino acids. Transmission electron micrograph of three Cd-treated genotypes revealed deformed chloroplast and mitochondrial membranes, and disfigured chloroplasts and irregularly shaped mitochondria.

It is suggested that differential response of B. juncea L. genotypes cannot be correlated to the amount of Cd accumulated in either of the organ but to the capability of the genotype to up-regulate the sulphur assimilation pathway, providing an enhanced supply of GSH for phytochelatin biosynthesis. Then, it might be the free amount of Cd that decides the magnitude of oxidative damage in the cell, as also evident from the ultra-structure of chloroplast and mitochondria of three Cd-treated genotypes (most susceptible, moderately susceptible and most resistant).

Keywords: *Brassica juncea* L. genotypes; differential Cd-sensitivity; sulphur metabolism; non-protein thiols; phytochelatins; chloroplast; mitochondrion.

Abbreviations: Cd: Cadmium; ROS: Reactive oxygen species; HSPs: Heat shock proteins; MTs: Metallothioneins; CDCA1: Cadmium-containing carbonic anhydraaase: NPTs: Non-protein thiols; PCs: Phytochelatins.

Introduction

Metals such as Iron (Fe), Zinc (Zn) Manganese (Mn), Molybedenom (Mo), Nickel (Ni) and Copper (Cu) are required for standard growth and development of plants. They serve as necessary cofactors for many biochemical enzymes and put up an essential structural roles in proteins. There are certain metals e.g., Cadmium (Cd) which are superfluous and have no known metabolism role in the life cycle of higher organisms as no enzyme has been identified which specifically requires Cd as a cofactor. However some non-traditional metal ions like Mg^{2+} have recently been shown to act as a secondary messenger and be credibly involved in cellular signaling and regulation of a number of metabolic pathways in living organisms [1]. Similarly Cd too has found its importance in the metabolism of a novel carbonic anhydrase (CDCA1) enzyme in the marine diatom. Thalassiosira weissflogii that naturally utilizes Cd^{2+} as catalytic metal ion, but if required can impulsively exchange Cd^{2+} to Zn^{2+} [2]. This does not negate to find its historic role as one of extremely hazardous environmental pollutant to animals, plants and other microbial life [3,4]. It assembles in the human body with a half-life 6-38 years and is responsible for a number of disorders like renal dysfunction [5-7], pulmonary emphysema [8], Osteotoxicity and multiple bone fractures [9,10], protein misfolding, aggregation of nascent/non native proteins, repoductive/developmental defects like damaged spermatogenesis and decreased motility of the sperms etc [10]. It has been found to act as a mitogen, boosts cancer in many organisms and has been classified as a proven human carcinogen [11,12]. In plants, it is responsible for a number of physiological malfunctioning [13,14], like targeting crucial biological macromoleucles [15], chlorosis, defected photosynthesis [16,17], lipid peroxidation [18,19], DNA damage [20], and even total crop failure [21,22]. A reduction in chlorophyll content upon treatment of plants with Cd has also been observed in a large number of plant species, suggesting that Cd disorganizes lamellar structures mainly the stroma and inhibits chlorophyll biosynthesis [23-25].

Brassica juncea is the third most important edible oil crop in the world. Besides, its seeds have other culinary uses in the preparation of pickles and curries. The whole plant has large ethonobotanical and industrial applications. The oil cake is used as animal feed and manure. The young leaves are used as salad, vegetables and are a good source of forage for cattle. Its ability to grow and flourish in poor and metal-contaminated soils has made it a potential crop for bioremediation. It exists an accurate source for gene manipulation and an ideal model to observe metal-tolerance mechanisms among the family Brassicaceae. Cd-tolerant *B.juncea* cultivars with high metal accumulation could be used in phytoremediation of Cd-contaminated soils, due to their high biomass and good soil binding property. The identification of such cultivars represents a potential tool to remediate the problems associated with Cd accumulation in terrestrial ecosystems. We have already reported the variation in phytoremediation potential of different *Brassica juncea* genotypes and have identified Cd-tolerant cultivars among them by studying their anti-oxidant mechanism both enzymatic and non-enzymatic [26]. The present study aims to analyze the secondary tolerance strategies which includes binding of Cd to phytochelatins, glutathione and aminoacids get altered under the Cd-stress in these *Brassica juncea* genotypes.

The effect of Cd on sulphur metabolism at the seedling stage on genotypes of the same species is hardly studied. Sulphur in its reduced state is found in essential biomolecules like amino-acids, proteins, in iron-sulfur clusters found in metalloproteins, α-lipoic acid, co-factors involved in electron transfer systems, though oxidized sulphur metabolites are also indispensable for plant metabolism existing as sulfonate group of modifying proteins, polysaccharides and lipids of various biological membranes. Sulphur, now being recognized as a fourth macronutrient also enhances the production of antioxidants that retaliates xenobiotic metabolism in the biological systems [27]. However, sulphur is relatively inert; it must be actively mobilised for further metabolism. Mobilistion in all cases studied requires the

key enzymes ATP-sulphurylase and adenosine 5'-phosphosulphate (APS) kinase. Further, ATP sulphurylase activity has been known since decades to increase during sulphur starvation [28]. No accumulation of mRNAs for ATP-sulphurylase has been shown in sulphur starved Arabidopsis thaliana along with the mRNA for sulphate permease [29].

Heavy metal toxicity induces a notable changes in sulphur metabolism of higher plants that accumulate heavy metal binding peptides termed as phytochelatins (PCs) [15]. Several heat shock proteins (HSP17, HSP70) have been found to induce and improve Cd-tolerance in living systems as observed in cell cultures of Cd- stressed Lycopersicon peruvianum [30], and in liver of zebrafish [31]. Earlier it was also reported that plants subjected to Cd stress get adapted to adverse effects by inducing HSPs of different molecular weights (HSPs; HSP100, HSP90, HSP70, HSP60) or HSP coagnates [32], and many of them have been well characterized in different crop species. These are in addition to selective stress responsive tripeptide glutathione that plays multidimensional roles in adapting to stressful environments.

Glutathione –a tripeptide with gamma peptide linkage, is the most abundant form of organic sulphur in plants, forming a major source of non-protein thiol, apart from playing countless roles in cellular metabolism [33,34]. As soon as Cd penetrates the cytoplasm of a cell, it alters sulphur metabolism pathways and diverts that into a system that lead to the formation of precursors of glutathjone (Glutamine, Cysteine and Glycine) responsible for the important complexing agents termed as phytochelatins [35,36]. Cadmium has a high affinity towards the activation of peptide ligands and has so far emerged as the best activator for the enzymes involved in the synthesis of PC synthase that interferes with the synthesise of PCs from .Phytochelatins form various complexes with Cd (with molecular masses of about 2500 or 3600), and restricts its circulation as free Cd^{2+} inside the cytoplasm [37].

The amount of accumulated amino acids in plants tissue are carefully regulated as they regulate ion transport, membrane permeability, and gene expression as well as enzyme activities. Moreover they are constituents of proteins, nucleic acid, osmolytes (proline) and other molecules (glutathione) that support growth and revolt oxidative stress in plants. Oxidative stress induced the activity of a set of cysteine proteases by post-translational mechanisms in soybean cells [38]. Heavy metals induces oxidative stress [39-41] and has a distinguished impact on protease activity in plants [42]. An increase in protease activity in the leaves of terrestrial (Vigna) as well as in aquatic (Hydrilla) plants with Cd treatment has been reported [43]. However, germinating seedlings Sorghum bicolor and rice showed a suppression of protease activities with Cd treatments leading to altered levels of proteins and amino acids [44,45]. It has been suggested that the extent of changes in proteases activity may vary with genotypes.

Because of these adverse effects of Cd on living systems, attempts are being made to minimize its level in the environment. The use of hyper accumulating plants to remove toxic Cd both from terrestrial and aquatic environments have been adapted as an ultimate solution to this trouble. This process of using several plants for environmental restoration by either removal or stabilizing the toxin is termed as phytomediation [46,47]. Cd is a particularly favorable target metal for this new technology because it is effortlessly transported and accumulated in distinct parts of many plant species. Despite a large body of literature regarding the ecology, evolution, genetics and physiology of plants adapted to growth on high concentrations of Cd, the physio-chemical mechanisms by which plant cells tolerate the toxic concentrations of Cd needs further investigations. Serious attempts are being made so as to find suitable plant species for removal of Cd from the contaminated environment. In this context we need a wide range of knowledge concerning the physiological and biochemical features of useful genotypes such as induction capability for osmolytes, efficiency of sulphur metabolism, strength of cellular antioxidants, expression threshold for antioxidants under Cd-stress and capability of Cd-detoxification or binding. The present study was carried out to examine;

(i) The differential accumulation of Cd in different organs of *B. juncea* L. genotypes,

(ii) The effect of Cd on components of sulphur metabolism, and

(iii) To evaluate the changes in chloroplast and mitochondrial ultrastructure to assess their role in plant defense against Cd Toxicity and specifically genotypic difference.

The results of this study could help to identify a potential hyperaccumulator genotype and engineering the others for greater for phytoremediation purpose.

Material and methods

Plant material, growth conditions and Experimental set up

The seeds of ten genotypes of *Brassica juncea.*, Vardhan (V1), Pusa Bahar (V2), Pusa Bold (V3), BTO (V4), Pusa Jai Kisan (V5), Agrini (V6), Varuna (V7), Kranti (V8), Vaibhav (V9) and Pusa Basant (V10) used for the study were obtained from National Research Centre on Plant Biotechnology, Indian Agricultural Research Institute (IARI), and New Delhi, Two day old Healthy and uniform germinating seedlings were shifted from paper towels to nutrient-rich solution with a water base [48], and were allowed to grow for a period of ten days. The solution was changed subsequently after every second day. After tenth day seedlings were treated with different concentrations of CdCl2 (T0, 0.0 mM; T1, 0.5 mM; T2, 1.0 mM; T3, 1.5 mM and T4, 2.0 mM) containing sterile nutrient solution. All the experiments were repeated thrice with three replicates. The mean (±SE) of values was presented in results. Seedlings were used to analyze the distribution and accumulation of cadmium in root, stem and leaf, whereas other parameters viz. phytochelatins, non-protein thiols, free amino acids, and activities of ATP-sulphurylase and proteases were studied in the leaf samples at 24, 48 and 72 hours after treatment (HAT).

Estimation of cadmium content and sample preparation

The seedlings were collected from the nutrient medium, washed with ultra-pure water and segregated into root, stem and leaves. The samples were dried out in a hot air oven at 65°C ± 2°C for 48 hrs. The powdered samples were digested in the Kjheldahl digestion assembly. 100 mg of the dried samples of root, stem and leaves were subjected to acid digestion [49,50]. The metal content (Cd) was estimated in parts per million (ppm) against the standard curve of CdCl2 by atomic absorption spectrophotometer (AAS; Video11, Thermo Jarrell Ash Corporation, USA) equipped with cathode lamp as an air-acetylene flame and was expressed as µg g-1 dry weight (dw) of the sample.

Soluble protein content

Soluble protein content was determined by Bradford's protein assay method [51]. The samples were calibrated against the standard curve of BSA (Sigma, Mo, USA). The protein content was expressed as mg g^{-1}fw.

Free amino acid pool

Soluble amino acid pool was prepared, extracted and evalvated spectrophotometrically at 570 nm [52] on uv-vis spectrophotometer (Model DU 640B, Beckman, USA), based on glycine (Sigma, Mo, USA) standard curve and expressed as mg g^{-1} fw.

***In vitro* protease activity**

In vitro protease activity was measured by reading the absorbance of samples prepared at 340 nm[53] on uv-vis spectrophotometer (Model DU 640, Beckman, USA). Protease activity was expressed in enzyme units as EU min-1g^{-1}fw.

Non-protein thiols (NPTs)

Non protein thiols were estimated by using Ellman's reagent [54]. A calibration curve was prepared using cysteine (Sigma, Mo, USA) to estimate non-protein thiols in samples. The result was expressed as nmol g^{-1} fresh weight (fw).

Phytochelatins (PCs)

The phytochelatins were calculated indirectly by subtracting the amount of glutathione from the amount of total non-protein thiols and result was expressed as nmol g^{-1} fw. PCs (nmol g^{-1}fw) = non-protein thiols – glutathione. Glutathione was estimated by the method of Anderson [55].

Estimation of *In vitro* ATP sulphurylase activity

For the assay of ATP-sulphurylase activity [56] absorbance was measured at 660 nm (Model DU 640, Beckman, USA). The enzyme activity was estimated against a standard curve of KH-2PO4 (10 to 100 (Mol) and was expressed as µmol Pi mg^{-1} protein min^{-1}

Transmission electron microscopy for ultrastructure of chloroplast and mitochondria

Small pieces (about 1-3 mm^2), of leaves of three genotypes were cut and stored in the fixing solution containing 1% formaldehyde, 2.5% glutaryldehyde and 2% paraformaldehyde, vacuum infiltrated for 10 min and fixed for overnight at 4°C. The samples were then rinsed with 0.1 M phosphate buffer (pH 7.4) and post fixed in osmium tetraoxide for 2 hrs at 4°C and then again rinsed with 0.1 M phosphate buffer (pH 7.4), and dehydrated with a graded series of acetone with increasing concentrations (30-90%) and finally in dry acetone (saturated with copper sulphate) for 1 h at 4°C. Dry acetone was exchanged with toluene twice for 60 min and placed in resin and toluene (1:3) for overnight in vacuum accompanied by impregnation in resin and toluene (2:2 and 3:1) for overnight in vacuum. Finally, samples were soaked in pure resin for 6 hrs at room temperature. Sections of the size of 500 nm were cut and stained in 1% methylene blue for 20-40 sec. Grids were made of the size of 60-90 nm and stained with heavy metal solution, uranyle acetate and lead citrate and these sections were then observed for transmission electron microscopy (Model CMIO, TEM, Phillips).

Statistical analysis

All the experiments were conducted out three times and the results of the study is here presented as the mean of three replicates. Values in text and tables indicate mean values ± SE and were subjected to two way ANOVA followed by Students' t test, taking $P \leq 0.05$ as significant.

Results

Cd- compartmentalisation and accumulation

The accumulation of Cd in the root, stem and leaf of all the *B. juncea* L. genotypes was found to be dose and time dependent. The Cd accumulation in roots was characterized by a high uptake of the metal. Maximum accumulation of Cd was observed in the root followed by stem in all the genotypes. Roots were found to accumulate 10 and 15 fold more Cd than shoots in V1 and V5, respectively (Table 1).

Cd-accumulation in the root varied from 25-41µg g^{-1} dw, 35-46 µg g^{-1} dw and 43-55µg g^{-1} dw, with the treatment T4 at 24, 48 and 72 HAT, respectively in *B. juncea* L genotypes. Similarly Cd-accumulation in shoots varied from 5.18-9.88µg g^{-1} dw, 7.74-11.88µg g^{-1} dw and 7.61-12.27 µg g^{-1} dw with the treatment T4 at 24, 48 and 72 HAT, respectively in *B. juncea* L genotypes. (Table 2)

Soluble protein

Leaf soluble protein increased in *B. juncea* L. genotypes with Cd-treatments at 24 and 48 HAT but declined thereafter. The increase varied from 6-24% at 24 HAT, and 9-25% at 48 HAT, respectively with T4, when compared with their respective control (Table 1). At 24 HAT, the increase in soluble protein was maximum in V5 (24%) and minimum in V8 (6%) when compared with the respective control. At 48 HAT, the increase in soluble protein was still maximum in V5 (25%) and minimum in V1 (9%) when compared with the respective control. At 72 HAT, the leaf soluble proteins, however, in these genotypes showed a marked decline over their respective control (Table 3). Minimum decline in soluble protein was observed in V5 (9%) and maximum in V1 (42%) with the treatment T4 when compared with the respective control. The differences in the soluble protein content among genotypes, Cd-treatments and their interactions were statistically significant at 5% level.

Soluble amino acids

The amount of soluble amino acids in Cd treated *B. juncea* L. genotypes increased and attained the maximum value by the end of experimental time (72 HAT). Amino acid levels in the leaves of V5 and V3 genotypes were less than V1 and V9, but still more than the respective control. At 24 HAT, the increase in amino acid level in these genotypes was only (11-37%) with the treatment T4, when compared with the respective control (Table 4). The increase in amino acid level was more, i.e. 18.6-47% and 20-58% at 48 and 72 HAT, respectively with T4 (2.0 mM), when compared with respective control. The differences in the level of amino acid among genotypes, Cd-treatments and their interactions were statistically significant at 5% level.

Protease activity

The activities of the acid proteases increased in a dose and time dependent manner, when seedlings of *B. juncea* L. genotypes were transferred to cadmium solution. The increase in the protease activity varied from 6-31%, 11-29% and 13-36%

with T4 at 24, 48 and 72 HAT, respectively in *Brassica juncea* L. genotypes as compared with their respective control. Minimum enhancement in protease activity was observed in V5 (13%) and maximum in V1 (36%) with T4 at 72 HAT when compared with respective control. These differences in protease activity were statistically significant when comparison was made among genotypes and Cd-treatments, while non-significant with respect to the interactions (Table 5).

Non protein thiol

Non protein thiols increased in all the *B. juncea* L. genotypes with all the Cd treatments. Increase in non-protein thiol content varied from 50-101%, 52-109% and 68-118% with T4 at 24, 48 and 72 HAT, respectively in *B. juncea* L. genotypes. Minimum increase in non-protein thiol was observed in V10 (68%) followed by V1 (66%), while maximum in V5 (118%) followed by V3 (96%) with T4 at 72 HAT, respectively over their control. The differences among genotypes, treatments and their interaction for non-protein thiols were statistically significant at 5% level. The data on non-protein thiols of *B. juncea* L. genotypes with varied concentration of cadmium chloride is presented in table 6.

Phytochelatins

Phytochelatins increased in all the *B. juncea* L. genotypes with the Cd stress. The increase in PC content varied from 57-140%, 57-145% and 31-150% with T4 at 24, 48 and 72 HAT, respectively among various genotypes. Maximum increase in phytochelatin content was observed in V5 (150%), followed by V3 (140%) with the Cd treatments T4 at 72 HAT, while minimum increase in V1 (31%), followed by V10 (59.5%) with the same treatment and at the same period, when compared with the respective control. The differences in the level of phytochelatins among genotypes, Cd-treatments and their interactions were statistically significant (Table 7).

ATP-Sulfurylase activity

ATP-sulphurylase activity increased in all the *B. juncea* L. genotypes with all the treatments. The increase varied from 6-18%, 11-25% and 17-32% with T4 at 24, 48 and 72 HAT, respectively among *B. juncea* L. genotypes as compared with their respective control (Table 8). The maximum ATP-sulphurylase activity was observed in V5 (32%), followed by V3 (34%), while minimum in V10¬ (17%) with T4 when compared with the control. These differences in ATP-sulphurylase activity among genotypes, Cd-treatments and genotypes X Cd-treatment interactions were statistically significant.

Cadmium application resulted into the loss of chloroplast integrity and subsequent decline in photosynthetic activity. In control, the chloroplasts were ellipsoidal, with a well-developed grana fretwork system and multi-thylakoid grana. Chloroplasts were smaller, of irregular shape, with a poorly developed grana fretwork, when treated with 2 mM CdCl2 (T4) for a period of 72 hours. The outer membranes as well as thylakoid membranes were disrupted in all the three *B. juncea* L. genotypes. However, the extent of damage was more in V1, (Figure 1) followed by V7 (Figure 2) and V5 (Figure 3). The similar pattern of damage was observed in mitochondrial morphology of these genotypes, under the treatments of same magnitude given for a period of 72 hrs (Figures 4,5,6).

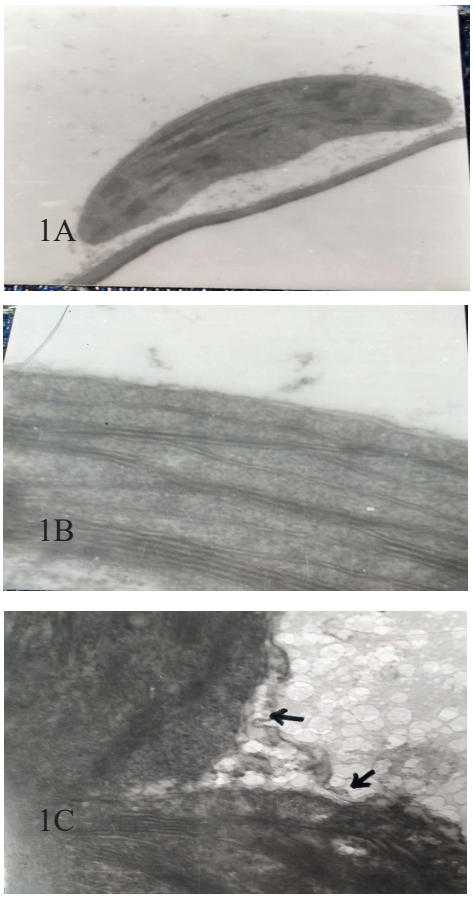

Figure 1: TEM images from chloroplast from the ten dayold leaf of Brassica juncea L.cv.Vardhan with 2 mM Cadmium chloride for 72 hrs.
A. Control undamaged chloroplast.
B. Control, a part magnified showing intact membrane and well organized thylakoids.
C Treated, apart magnified showing membrane disruption and disappearance of thylakoids (Arrow indicate the damaged areas).

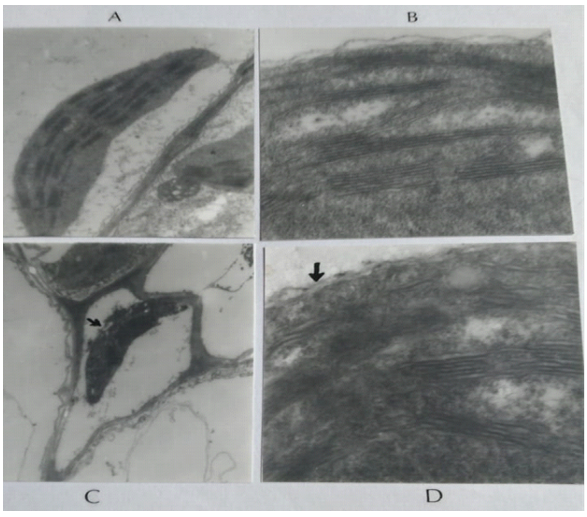

Figure 2: TETEM images from chloroplast from the ten day old leaf of Brassica juncea L.cv.Varruna with 2 mM Cadmium chloride for 72 hrs.
A. Control undamaged chloroplast.
B. Control, a part magnified showing intact membrane and well organized thylakoids.
C Treated, showing damaged chloroplast.
D Treated, apart magnified showing membrane disruption and disappearance of thylakoids (Arrow indicate the damaged areas).

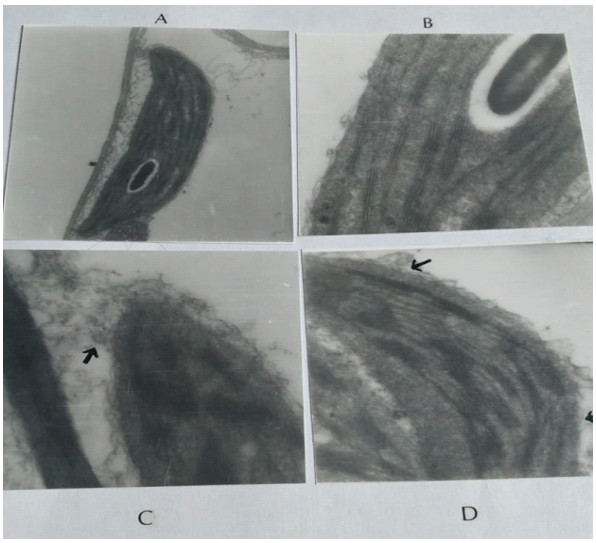

Figure 3: TEM images from chloroplast from the ten day old leaf of Brassica juncea L.cv.pusa jai kisan with 2 mM Cadmium chloride for 72 hrs.
A. Control undamaged chloroplast.
B. Control, a part magnified showing intact membrane and well organized thylakoids.
C Treated, showing damaged chloroplast.
D Treated, apart magnified showing membrane disruption and disappearance of thylakoids (Arrow indicate the damaged areas).

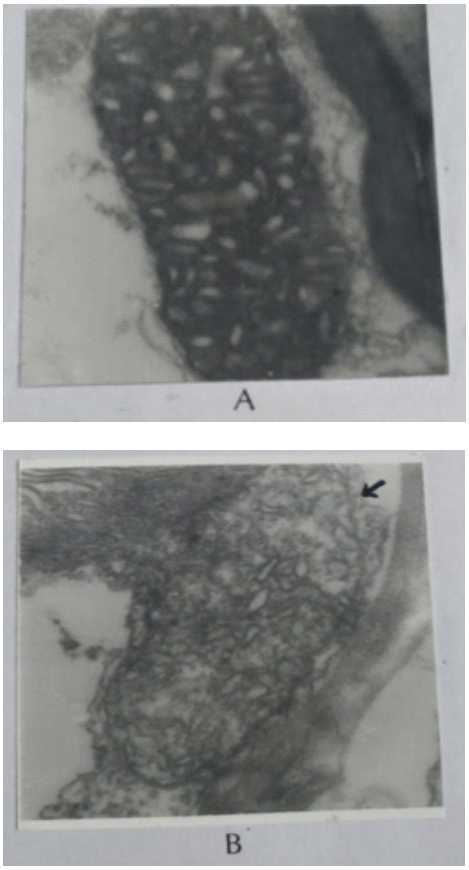

Figure 4: TEM images from of mitochondria from the ten day old leaf of Brassica juncea L.cv.Vardhan with 2 mM Cadmium chloride for 72 hrs.
A. Control Showing undamaged mitochondria (The normal mitochondrial electron dense matrix surround the intact cristae).
B. Showing damaged mitochondria (The mitochondrial cristae are fragmented and ramnants of damaged cristae are visible).

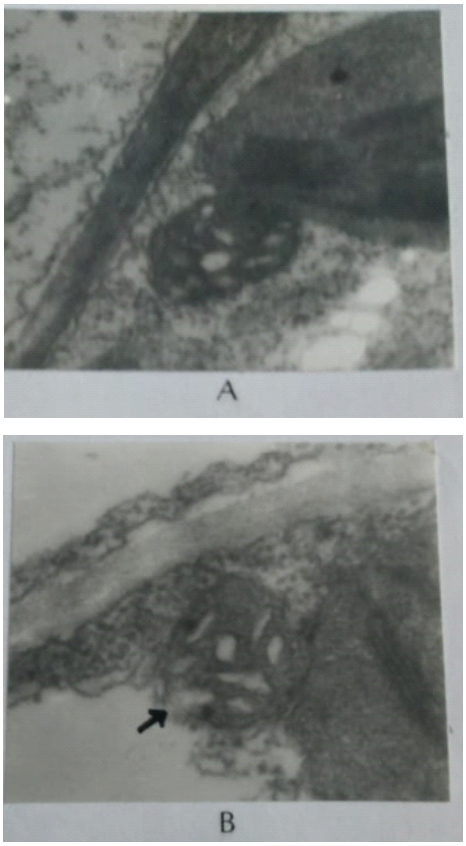

Figure 5: TEM images from mitochondria from the ten day old leaf of *Brassica juncea* L.cv.Varuna with 2 mM Cadmium chloride for 72 hrs.
A. Control Showing undamaged mitochondria where regular array of mitochondrial cristae are seen with organized membrane.
B. Showng damaged mitochondria showing disorganized outer as well as inner membrane and initiation of the loss of cristae.

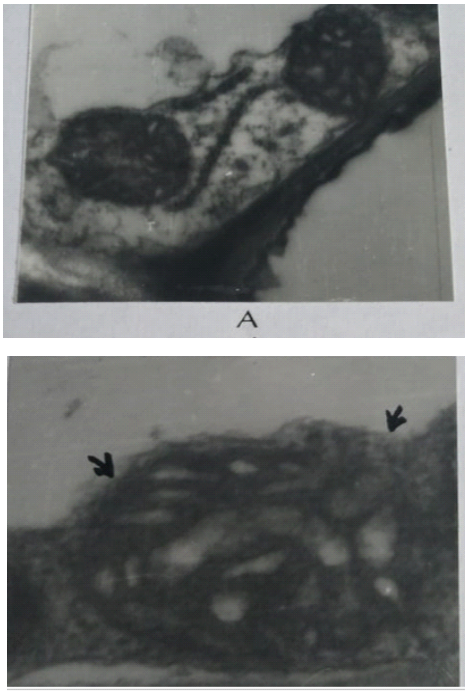

Figure 6: TEM images from mitochondria from the ten day old leaf of Brassica juncea L.cv. pusa jai kisan with 2 mM Cadmium chloride for 72 hrs.
A Control Showing undamaged mitochondria where regular array of mitochondrial cristae are seen.
B. Showng a little damaged mitochondria where slightly disrupted mitochondrial membrane can be seen.

Table 1: Variations in Cd- accumulation in the root (µg g^{-1} dw) of *B. juncea* L. genotypes under Cd-stress.

Genotypes	Treatment (mM CdCl2)				
24HAT	**T_0**	**T_1**	**T_2**	**T_3**	**T_4**
V_1	0.0 ± 0.0	7.33 ± 0.31	12.67 ± 0.42	23.67 ± 0.16	25.33 ± 1.29
V_2	0.0 ± 0.0	15.31 ± 0.59	19.33 ± 0.20	20.79 ± 0.41	29.04 ± 0.23
V_3	0.0 ± 0.0	24.33 ± 0.42	28.67 ± 0.16	32.67 ± 0.46	36.33 ± 0.68
V_4	0.0 ± 0.0	9.33 ± 0.16	21.67 ± 0.42	36.67 ± 0.42	41.33 ± 0.63
V_5	0.0 ± 0.0	16.00 ± 0.72	20.67 ± 0.57	33.67 ± 0.42	41.00 ± 0.27
V_6	0.0 ± 0.0	8.00 ± 0.27	24.33 ± 0.57	25.67 ± 0.57	31.67 ± 0.42
V_7	0.0 ± 0.0	11.73 ± 0.42	19.00 ± 0.27	26.33 ± 0.42	35.33 ± 0.16
V_8	0.0 ± 0.0	12.33 ± 0.42	17.00 ± 0.11	26.67 ± 0.16	26.67 ± 0.57
V_9	0.0 ± 0.0	8.33 ± 0.16	14.00 ± 0.54	24.67 ± 0.87	31.00 ± 0.27
V_{10}	0.0 ± 0.0	12.27 ± 0.17	14.77 ± 0.11	26.67 ± 0.16	28.00 ± 0.27
48HAT)V_1	0.0 ± 0.0	14.33 ±0.16	38.33 ± 0.16	42.33 ±0.79	45.83 ± 0.35
V_2	0.0 ± 0.0	25.67 ± 0.31	29.00 ± 0.27	37.00 ±0.54	42.00 ± 0.27
V_3	0.0 ± 0.0	24.67 ± 0.31	29.67 ± 0.31	33.33 ± 0.79	35.33 ± 1.10
V_4	0.0 ± 0.0	23.33 ± 0.42	33.67 ± 0.16	44.00 ± 0.72	46.67 ± 0.87
V_5	0.0 ± 0.0	28.00 ± 1.25	39.33 ± 0.57	44.67 ± 0.68	44.67 ±0.68
V_6	0.0 ± 0.0	18.00 ± 0.27	32.00 ± 0.54	40.33 ± 0.83	45.33 ± 0.68
V_7	0.0 ± 0.0	25.33 ± 0.42	28.00 ± 0.27	34.67 ± 0.68	40.00 ± 0.27
V_8	0.0 ± 0.0	18.67 ± 0.42	34.67 ±0.16	38.00 ± 0.27	45.67 ± 0.54
V_9	0.0 ± 0.0	20.33 ± 0.57	33.67 ± 0.79	44.33 ± 1.10	44.00 ± 0.27
72HAT V_{10}	0.0 ± 0.0	28.33 ± 0.31	36.33 ± 0.33	37.33 ± 1.75	44.00 ± 0.47
V_1	0.0 ± 0.0	28.33 ± 0.31	40.77 ± 0.29	43.33 ± 0.79	46.00 ± 0.27
V_2	0.0 ± 0.0	35.00 ± 0.54	37.00 ± 0.54	40.67 ± 0.57	43..57 ± 0.68
V_3	0.0 ± 0.0	35.00 ± 0.27	45.00 ± 0.72	48.00 ± 0.27	51.00 ± 0.27
V_4	0.0 ± 0.0	33.00 ± 0.54	46.00 ± 0.47	45.67 ± 0.42	49.67 ± 0.16
V_5	0.0 ± 0.0	34.00 ± 0.72	43.33 ± 0.42	52.33 ± 0.68	55.67 ± 0.31
V_6	0.0 ± 0.0	33.00 ± 0.47	39.00± 0.02	43.00 ± 0.42	46.00 ± 0.82
V_7	0.0 ± 0.0	33.33 ± 0.42	40.33 ± 0.57	52.33 ± 0.31	54.33 ± 0.68
V_8	0.0 ± 0.0	26.00 ± 0.27	38.00 ± 0.27	43.33 ± 0.57	47.00 ± 0.54
V_9	0.0 ± 0.0	33.00 ± 0.47	47.00 ± 0.54	51.00 ± 0.72	51.00 ± 0.27
V_{10}	0.0 ± 0.0	33.00 ± 0.98	37.00 ± 0.94	44.00 ± 0.72	44.67 ± 0.68
CD at 5%:		**24HAT**	**48HAT**	72HAT	
Genotypes*		**1.1098**	**1.5754**	**1.3085**	
Treatments*		**0.7848**	**1.444**	**0.9253**	
Genotypes x Treatments*		**2.4817**	**3.5227**	**2.926**	

Table 2: Variations in Cd accumulation in the Shoot (µg g^{-1} dw) of *B. juncea* L. genotypes under Cd-stress.

Genotypes	Treatment (mM CdCl2)				
24HAT	**T_0**	**T_1**	**T_2**	**T_3**	**T_4**
V_1	0.0 ± 0.0	2.59 ± 0.05	3.27 ± 0.07	5.26± 0.10	5.18± 0.16
V_2	0.0 ± 0.0	5.05 ± 0.16	5.75± 0.16	6.84 ± 0.42	7.17± 0.27
V_3	0.0 ± 0.0	4.57 ± 0.05	7.83 ± 0.21	9.13 ± 0.27	9.33± 0.17
V_4	0.0 ± 0.0	4.23 ± 0.16	6.70 ± 0.08	9.95 ± 0.10	9.11 ± 0.08
V_5	0.0 ± 0.0	5.59 ± 0.07	5.96 ±0.07	7.86 ± 0.27	9.88 ± 0.27
V_6	0.0 ± 0.0	4.90 ± 0.07	5.73 ± 0.10	7.87 ± 0.04	7.93 ± 0.09
V_7	0.0 ± 0.0	4.83 ± 0.27	7.33 ± 0.16	7.55 ± 0.25	7.61 ± 0.09
V_8	0.0 ± 0.0	3.73 ± 0.21	4.05± 0.01	6.37 ± 0.15	7.42 ± 0.34
V_9	0.0 ± 0.0	2.26 ± 0.00	4.53 ± 00.07	6.77± 0.05	7.52 ± 0.19
V_{10}	0.0 ± 0.0	3.87 ± 0.10	4.80 ± 0.07	6.35 ± 0.07	4.44 ± 0.10
48 HAT V_1	0.0 ± 0.0	3.45 ± 0.07	5.10± 0.08	5.51 ± 0.08	7.74 ± 0.04
V_2	0.0 ± 0.0	6.97 ± 0.07	7.26± 0.14	8.72 ± 0.03	8.82 ± 0.08
V_3	0.0 ± 0.0	8.43 ± 0.19	10.30± 0.21	12.00 ± 0.12	12.40± 0.10
V_4	0.0 ± 0.0	8.35 ± 0.09	9.68 ± 0.15	11.10 ± 0.07	11.83 ± 0.04
V_5	0.0 ± 0.0	7.93 ± 0.07	10.00± 0.14	11.18 ± 0.28	11.88± 0.08
V_6	0.0 ± 0.0	8.04 ± 0.06	8.73 ± 0.19	9.47± 0.18	10.30 ± 0.26
V_7	0.0 ± 0.0	7.87 ± 0.08	8.65± 0.22	11.35 ± 0.23	11.65 ± 0.07
V_8	0.0 ± 0.0	5.18 ± 0.09	7.75 ±0.16	8.00 ± 0.10	9.37± 0.11
V_9	0.0 ± 0.0	4.74 ± 0.14	6.37 ± 0.10	7.31± 0.03	9.08 ± 0.17
V_{10}	0.0 ± 0.0	6.27 ± 0.10	6.63± 0.01	8.36 ± 0.10	9.58 ± 0.13
72HAT V_1	0.0 ± 0.0	4.27 ± 0.15	5.01± 0.09	5.86 ± 0.14	7.61±0.10
V_2	0.0 ± 0.0	7.20± 0.03	7.80 ± 0.03	9.63 ± 0.03	10.23 ± 0.07
V_3	0.0 ± 0.0	9.21± 0.17	12.06 ± 0.12	11.90 ± 0.10	12.92 ± 0.10
V_4	0.0 ± 0.0	9.50± 0.21	10.90 ± 0.24	10.87 ± 0.16	12.20 ± 0.12
V_5	0.0 ± 0.0	8.63 ± 0.22	10.36 ± 0.08	12.31± 0.29	12.27± 0.34
V_6	0.0 ± 0.0	7.63 ± 0.10	10.03 ± 0.07	9.80 ± 0.08	10.96± 0.09
V_7	0.0 ± 0.0	9.80 ± 0.10	10.28 ± 0.13	10.93 ± 0.10	11.27± 0.15
V_8	0.0 ± 0.0	5.99 ±0.09	3.38 ± 0.09	3.27 ± 0.14	4.37 ±0.14
V_9	0.0 ± 0.0	5.06 ±0.07	2.62 ± 0.05	3.20 ± 0.02	4.28 ± 0.07
V_{10}	0.0 ± 0.0	6.59± 0.08	2.73 ± 0.06	3.89 ± 0.03	3.95 ± 0.07
CD at 5%:		**24HAT**	**48HAT**	**72HAT**	
Genotypes*		**0.3951**	**0.326**	**0.3228**	
Treatments*		**0.2794**	**0.2306**	**0.2282**	
Genotypes x Treatments*		**0.8835**	**0.7291**	**0.7218**	

Table 3: Variations in soluble protein content (mg g^{-1} fw) of *B. juncea* L. genotypes under Cd-stress.

Genotypes	Treatment (mM CdCl2)				
24HAT	**T_0**	**T_1**	**T_2**	**T_3**	**T_4**
V_1	6.39 ± 0.01	6.55 ± 0.15	6.73 ± 0.14	6.83 ± 0.14	6.83 ± 0.18
V_2	7.63 ± 0.12	8.29 ± 0.02	8.36 ± 0.09	8.41 ± 0.14	8.71 ± 0.10
V_3	7.23 ± 0.14	7.49 ± 0.12	8.06 ± 0.06	8.28 ± 0.07	8.60 ± 0.05
V_4	7.53 ± 0.14	8.20 ± 0.06	8.87 ± 0.13	9.00 ± 0.06	9.12 ± 0.05
V_5	7.19 ± 0.19	7.34 ± 0.08	7.48 ± 0.10	7.74 ± 0.09	8.90 ± 0.02
V_6	7.09 ± 0.09	7.28 ± 0.04	7.34 ± 0.07	7.98 ± .001	8.13 ± 0.18
V_7	7.87 ± 0.08	8.25 ± 0.07	8.38 ± 0.09	8.65 ± 0.13	8.66 ± 0.08
V_8	7.84 ± 0.15	7.91 ± 0.08	7.95 ± 0.03	8.14 ± 0.03	8.28 ± 0.04
V_9	6.58 ± 0.08	6.62 ± 0.08	6.75 ± 0.07	6.98 ± 0.04	7.13 ± 0.03
V_{10}	5.38 ± 0.09	5.53 ± 0.06	5.76 ± 0.07	5.85 ± 0.10	6.01 ± 0.04
48HAT V_1	6.75 ± 0.04	6.90 ± 0.15	7.33 ± 0.15	7.35 ± 0.04	7.35 ± 0.11
V_2	7.33 ± 0.03	8.49 ± 0.13	8.64 ± 0.05	8.70 ± 0.11	8.97 ± 0.13
V_3	7.30 ± 0.08	7.88 ± 0.06	8.23 ± 0.08	8.41 ± 0.02	8.86 ± 0.03
V_4	7.91 ± 0.15	8.87 ± 0.02	9.37 ± 0.09	9.10 ± 0.20	9.43 ± 0.12
V_5	8.06 ± 0.04	8.61 ± 0.11	8.96 ± 0.08	9.09 ± 0.16	10.06 ± 0.05
V_6	7.86 ± 0.13	8.68 ± 0.06	8.80 ± 0.06	8.82 ± 0.08	8.84 ± 0.04
V_7	7.97 ± 0.01	8.43 ± 0.13	8.45 ± 0.22	8.93 ± 0.07	8.99 ± 0.12
V_8	8.46 ± 0.12	9.45 ± 0.08	9.47 ± 0.06	9.50 ± 0.02	9.50 ± 0.03
V_9	6.54 ± 0.10	7.24 ± 0.02	7.28 ± 0.04	7.32 ± 0.05	7.57 ± 0.03
V_{10}	5.47 ± 0.06	6.04 ± 0.07	6.01 ± 0.02	6.02 ± 0.01	6.07 ± 0.03
72 HAT V_1	7.90 ± 0.13	6.32 ± 0.05	6.16 ± 0.17	6.05 ± 0.12	4.59 ± 0.09
V_2	8.76 ± 0.11	8.29 ± 0.04	8.14 ± 0.05	7.80 ± 0.01	6.80 ± 0.10
V_3	7.74 ± 0.10	7.23 ± 0.02	7.17 ± 0.04	7.17 ± 0.07	7.06 ± 0.13
V_4	8.29 ± 0.09	7.86 ± 0.25	7.70 ± 0.09	7.56 ± 0.13	6.93 ± 0.26
V_5	8.90 ± 0.12	8.79 ± 0.04	8.20 ± 0.08	8.18 ± 0.17	8.09 ± 0.06
V_6	9.04 ± 0.02	8.45 ± 0.07	7.90 ± 0.07	7.35 ± 0.08	7.03 ± 0.01
V_7	9.21 ± 0.03	8.72 ± 0.16	7.62 ± 0.16	7.59 ± 0.11	7.31 ± 0.01
V_8	8.59 ± 0.07	7.93 ± 0.05	7.65 ± 0.16	7.27 ± 0.04	6.15 ± 0.05
V_9	7.44 ± 0.12	6.73 ± 0.10	6.71 ± 0.07	6.56 ± 0.07	4.67 ± 0.05
V_{10}	6.23 ± 0.06	5.64 ± 0.08	5.53 ± 0.09	5.40 ± 0.14	4.44 ± 0.12
CD at 5%:		**24HAT**	**48HAT**	72HAT	
Genotypes*		**0.2528**	**0.2398**	**0.2813**	
Treatments*		**0.1787**	**0.1696**	**0.1989**	
Genotypes x Treatments*		**0.5652**	**0.5363**	**0.629**	

Table 4: Variations in soluble Amino acid content (mg g^{-1} fw) of B. juncea L. genotypes under Cd-stress.

24HAT(Genotypes)	(treatment)T_0	T_1	T_2	T_3	T_4
V_1	0.810 ± 0.04	0.903 ± 0.03	0.913 ± 0.04	0.930 ± 0.22	1.047 ± .05
V_2	0.797 ± 0.04	0.843 ± 0.04	0.836 ± 0.05	0.990 ± 0.14	1.090 ± 0.14
V_3	1.017 ± 0.05	1.133 ± 0.05	1.180 ± 0.07	1.253 ± 0.06	1.260 ± 0.06
V_4	1.060 ± 0.06	1.083 ± 0.07	0.203 ± 0.03	1.257 ± 0.09	1.297 ± 0.08
V_5	1.050 ± 0.03	1.093 ± 0.06	1.097 ± 0.04	1.160 ± 0.04	1.163 ± 0.06
V_6	0.780 ± 0.04	0.837 ± 0.05	0.833 ± 0.04	0.870 ± 0.04	0.923 ± 0.05
V_7	0.727 ± 0.04	0.787 ± 0.03	0.803 ± 0.04	0.837 ± 0.04	0.847 ± 0.03
V_8	1.207 ± 0.06	1.273 ± 0.04	1.303 ± 0.08	1.307 ± 0.08	1.353 ± 0.05
V_9	0.970 ± 0.04	0.980 ± 0.04	1.15 ± 0.04	1.173 ± 0.06	1.180 ± 0.02
V_{10}	0.887 ± 0.02	0.957 ± 0.02	1.042 ± 0.15	1.087 ± 0.04	1.023 ± 0.04
48HAT V_1	0.903 ± 0.04	1.113 ± 0.07	1.163 ± 0.03	1.187 ± 0.02	1.330 ± 0.06
V_2	0.843 ± 0.04	0.940 ± 0.03	0.983 ± 0.07	1.123 ± 0.08	1.130 ± 0.08
V_3	1.143 ± 0.08	1.257 ± 0.04	1.377 ± 0.05	1.343 ± 0.08	1.357 ± 0.09
V_4	1.117 ± 0.08	1.170 ± 0.07	1.303 ± 0.07	1.507 ± 0.03	1.613 ± 0.05
V_5	1.133 ± 0.04	1.217 ± 0.04	1.243 ± 0.05	1.343 ± 0.044	1.467 ± 0.07
V_6	0.923 ± 0.049	0.990 ± 0.05	1.177 ± 0.07	1.182 ± 0.082	1.198 ± 0.08
V_7	0.830 ± 0.04	0.897 ± 0.128	1.017 ± 0.03	1.077 ± 0.08	1.183 ± 0.02
V_8	1.223 ± 0.07	1.370 ± 0.09	1.413 ± 0.06	1.432 ± 0.03	1.607 ± 0.05
V_9	1.047 ± 0.06	1.263 ± 0.052	1.340 ± 0.09	1.500 ± 0.06	1.510 ± 0.100
V_{10}	1.013 ± 0.02	1.107 ± 0.05	1.277 ± 0.06	1.307 ± 0.09	1.223 ± 0.01
72HAT V_1	1.183 ± 0.04	1.350 ± 0.05	1.483 ± 0.02	1.500 ± 0.07	1.873 ± .007
V_2	0.977 ± 0.04	1.093 ± 0.09	1.143 ± 0.08	1.293 ± 0.06	1.477 ± 0.02
V_3	1.203 ± 0.04	1.357 ± 0.04	1.490 ± 0.07	1.570 ± 0.07	1.570 ± 0.05
V_4	1.153 ± 0.03	1.210 ± 0.08	1.397 ± 0.08	1.463 ± 0.07	1.617 ± 0.08
V_5	1.263 ± 0.02	1.437 ± 0.03	1.397 ± 0.06	1.507 ± 0.05	1.523 ± 0.01
V_6	1.000 ± 0.07	1.190 ± 0.06	1.293 ± 0.07	1.317 ± 0.07	1.537 ± 0.02
V_7	0.973 ± 0.07	1.140 ± 0.34	1.160 ± 0.05	1.193 ± 0.07	1.373 ± 0.02
V_8	1.250 ± 0.05	1.440 ± 0.05	1.443 ± 0.12	1.530 ± 0.05	1.947 ± 0.02
V_9	1.207 ± 0.06	1.473 ± 0.05	1.473 ± 0.06	1.520 ± 0.05	1.907 ± 0.01
V_{10}	1.170 ± 0.04	1.350 ± 0.04	1.470 ± 0.05	1.537 ± 0.05	1.877 ± 0.02
CD at 5%:		**24HAT**	**48HAT**	**72HAT**	
Genotypes*		**0.1501**	**0.1706**	**0.1365**	
Treatments*		**0.1062**	**0.1206**	**0.0965**	
Genotypes x Treatments*		**NS (Not significant)**	**NS (Not significant)**	**0.629**	

Table 5: Variations in protease activity (U min-1 g-1 fw) of *B. juncea* L. genotypes under Cd-stress.

Genotypes	Treatment (mM CdCl2)				
24HAT	**T_0**	**T_1**	**T_2**	**T_3**	**T_4**
V_1	1.72 ± 0.01	1.77 ± 0.005	1.82 ± 0.007	1.982 ± 0.006	2.24 ± 0.138
V_2	2.15 ± 0.008	2.20 ± 0.008	2.24 ± 0.008	2.44 ± 0.030	2.42 ± 0.061
V_3	0.83 ± 0.008	0.88 ± 0.004	0.89 ± 0.006	0.90 ± 0.007	0.94 ± 0.008
V_4	1.54 ± 0.009	1.58 ± 0.007	1.65 ± 0.007	1.73 ± 0.009	1.73 ± 0.009
V_5	1.75 ± 0.008	1.78 ± 0.036	1.80 ± 0.043	1.86 ± 0.076	1.86 ± 0.065
V_6	1.17 ± 0.007	1.24 ± 0.008	1.28 ± 0.007	1.35 ± 0.054	1.36 ± 0.005
V_7	1.70 ± 0.030	1.73 ± 0.050	1.80 ± 0.042	1.86 ± 0.058	1.95 ± 0.054
V_8	1.48 ± 0.039	1.59 ± 0.061	1.60 ± 0.016	1.66 ± 0.013	1.72 ± 0.010
V_9	0.85 ± 0.005	0.91 ± 0.005	0.97 ± 0.012	0.98 ± 0.010	0.99 ± 0.008
V_{10}	1.28 ± 0.016	1.32 ± 0.013	1.33 ± 0.011	1.44 ± 0.116	1.46 ± 0.013
48HAT V_1	1.78 ± 0.028	1.82 ± 0.050	1.88 ± 0.053	1.95 ± 0.070	2.27 ± 0.016
V_2	2.21 ± 0.057	2.41 ± 0.028	2.69 ± 0.044	2.72 ± 0.0507	2.76 ± 0.014
V_3	1.01 ± 0.050	1.02 ± 0.010	1.06 ± 0.016	1.14 ± 0.013	1.16 ± 0.016
V_4	1.55 ± 0.062	1.61 ± 0.073	1.80 ± 0.043	1.78 ± 0.065	1.94 ± 0.084
V_5	1.71 ± 0.087	1.73 ± 0.040	1.85 ± 0.054	1.89 ± 0.033	1.89 ± 0.038
V_6	1.33 ± 0.082	1.37 ± 0.010	1.44 ± 0.010	1.50 ± 0.013	1.56 ± 0.013
V_7	1.76 ± 0.015	1.84 ± 0.016	1.97 ± 0.019	1.98 ± 0.091	2.10 ± 0.090
V_8	1.49 ± 0.047	1.61 ± 0.022	1.72 ± 0.047	1.76 ± 0.036	2.10 ± 0.016
V_9	0.93 ± 0.041	0.99 ± 0.014	1.02 ± 0.010	1.05 ± 0.062	1.93 ± 0.062
V_{10}	1.36 ± 0.010	1.40 ± 0.030	1.41 ± 0.043	1.48 ± 0.054	1.64 ± 0.103
72HAT V_1	1.87 ± 0.071	1.93 ± 0.084	2.18 ± 0.089	2.43 ± 0.021	2.54 ± 0.022
V_2	2.35 ± 0.080	2.56 ± 0.077	2.87 ± 0.035	2.88 ± 0.051	2.86 ± 0.070
V_3	1.03 ± 0.017	1.12 ± 0.035	1.16 ± 0.017	1.19 ± 0.050	1.21 ± 0.05
V_4	1.71 ± 0.032	1.90 ± 0.093	1.99 ± 0.043	1.99 ± 0.055	2.08 ± 0.02
V_5	1.84 ± 0.096	1.79 ± 0.068	1.94 ± 0.077	1.96 ± 0.099	2.09 ± 0.027
V_6	1.53 ± 0.033	1.67 ± 0.012	1.79 ± 0.052	1.87 ± 0.032	1.91 ± 0.037
V_7	1.92 ± 0.025	1.67 ± 0.052	2.01 ± 0.052	2.18 ± 0.032	2.26 ± 0.096
V_8	1.86 ± 0.026	1.97 ± 0.052	2.06 ± 0.068	2.07 ± 0.062	2.13 ± 0.053
V_9	0.96 ± 0.030	1.02 ± 0.035	1.08 ± 0.045	1.15 ± 0.042	1.26 ± 0.042
V_{10}	1.38 ± 0.063	1.53 ± 0.027	1.59 ± 0.077	1.67 ± 0.036	1.79 ± 0.041
CD at 5%:		**24HAT**	**48HAT**	**72HAT**	
Genotypes*		**0.0922**	**0.082**	**0.1397**	
Treatments*		**0.0652**	**0.058**	**0.0988**	
Genotypes x Treatments*		**NS**	**NS**	**NS**	

Table 6: Variations in Non protein thiol (NPT) content (nmol g-1 fw) of B. juncea L. genotypes under Cd-stress

Genotypes	Treatment (mM CdCl2)				
24HAT	**T_0**	**T_1**	**T_2**	**T_3**	**T_4**
V_1	66.84 ± 2.01	74.38 ± 1.75	82.46 ± 0.31	89.66 ± 0.33	100.33 ± 0.87
V_2	73.15 ± 1.32	89.22 ± 0.18	109.67 ± 0.57	118.39 ± 0.46	125.05 ± 0.52
V_3	90.47 ± 0.41	100.28 ± 1.15	112.70 ± 0.36	139.04 ± 0.87	164.02 ± 0.54
V_4	75.33 ± 1.25	88.50 ± 0.25	108.87 ± 0.70	127.75 ± 0.64	143.00 ± 0.54
V_5	80.99 ± 0.77	103.66 ± 0.89	117.72 ± 1.21	137.76 ± 0.64	162.67 ± 0.68
V_6	77.78 ± 1.30	87.52 ± 0.28	97.95 ± 0.20	107.92 ± 1.14	137.00 ± 0.72
V_7	77.94 ± 1.30	94.82 ± 0.83	105.84 ± 0.16	121.06 ± 0.52	143.00 ± 0.98
V_8	78.32 ± 0.55	85.78 ± 0.46	95.81 ± 0.15	107.26 ± 1.47	125.42 ± 1.32
V_9	73.50 ± 1.13	84.84 ± 0.96	95.32 ± 0.68	108.82 ± 0.32	111.33 ± 0.87
V_{10}	69.54 ± 1.65	80.91 ± 0.23	91.72 ± 0.36	92.82 ± 0.52	105.67 ± 0.83
48HAT V_1	76.53 ± 0.28	89.20 ± 0.33	100.85 ± 0.42	108.65 ± 0.95	116.43 ± 0.40
V_2	92.40 ± 0.44	107.58 ± 0.57	126.18 ± 0.64	135.31 ± 0.88	158.82 ± 0.40
V_3	99.81 ± 1.03	118.08 ± 0.08	148.21 ± 0.43	166.57 ± 0.65	184.42 ± 0.32
V_4	88.27 ± 0.94	105.09 ± 1.15	122.82 ± 1.03	135.64 ± 0.61	162.65 ± 0.50
V_5	88.41 ± 0.48	115.17 ± 1.76	141.38 ± 1.00	160.50 ± 0.79	184.88 ± 0.12
V_6	85.14 ± 0.26	108.74 ± 1.15	128.91 ± 1.15	136.72 ± 0.78	147.37 ± 0.37
V_7	96.77 ± 0.48	107.31 ± 0.98	119.49 ± 1.23	148.35 ± 0.30	172.98 ± 1.80
V_8	92.63 ± 0.23	107.31 ± 0.98	123.85 ± 1.11	137.14 ± 0.36	152.07 ± 0.70
V_9	81.95 ± 0.46	107.51 ± 0.80	112.91 ± 0.89	119.59 ± 0.19	126.97 ± 0.90
V_{10}	76.41 ± 0.55	97.64 ± 1.32	102.31 ± 0.64	115.65 ± 0.48	121.49 ± 0.54
V_1	87.12 ± 0.27	106.65 ± 0.82	120.68 ± 0.30	135.47 ± 0.14	144.47 ± 0.45
72HAT V_2	95.35 ± 0.31	127.71 ± 0.43	143.58 ± 0.27	160.66 ± 0.62	173.11 ± 0.27
V_3	107.16 ± 0.42	144.51 ± 0.74	168.30 ± 0.77	186.75 ± 0.09	210.17 ± 0.35
V_4	95.29 ± 0.19	118.34 ± 0.51	146.67 ± 0.36	172.51 ± 0.19	182.17 ± 0.45
V_5	98.29 ± 0.27	142.85 ± 0.76	174.66 ± 1.87	209.18 ± 0.34	214.81 ± 0.41
V_6	96.46 ± 0.56	118.50 ± 0.69	134.99 ± 0.77	157.79 ± 0.26	175.93 ± 0.36
V_7	103.44 ± 0.21	132.58 ± 0.77	142.18 ± 0.42	188.84 ± 0.33	190.38 ± 0.41
V_8	104.32 ± 0.21	126.04 ± 0.48	150.77 ± 0.71	175.91 ± 0.69	177.49 ± 0.17
V_9	94.43 ± 0.25	129.58 ± 0.27	137.91 ± 0.42	148.54 ± 0.14	158.01 ± 0.47
V_{10}	88.54 ± 0.12	116.62 ± 0.19	125.11 ± 0.29	143.78 ± 0.27	149.33 ± 0.20
CD at 5%:		**24HAT**	**48HAT**	**72HAT**	
Genotypes*		**2.3924**	**2.1321**	**3.9133**	
Treatments*		**1.6917**	**1.5076**	**2.7672**	
Genotypes x Treatments*		**5.3496**	**4.7675**	**7.7505**	

Table 7: Variations in phytochelatin (SH-PC) content (nmol g^{-1} fw) of *B. juncea* L. genotypes under Cd-stress.

Genotypes	Treatment (mM CdCl2)				
24HAT	**T_0**	**T_1**	**T_2**	**T_3**	**T_4**
V_1	12.00 ± .957	14.16 ± 1.24	16.57 ± 0.79	17.36 ± 0.84	18.95 ± 0.32
V_2	11.02 ± 0.10	15.97 ± 0.72	18.80 ± 0.33	24.05 ± 0.30	25.46 ± 1.70
V_3	18.72 ± 1.58	20.50 ± 0.65	24.40 ± 0.72	35.87 ± 0.67	43.73 ± 1.43
V_4	20.64 ± 1.42	28.14 ± 1.32	39.37 ± 0.43	46.00 ± 0.76	47.99 ± 1.05
V_5	25.00 ± 1.00	32.77 ± 1.12	40.16 ± 0.71	54.52 ± 0.80	60.07 ± 1.88
V_6	12.98 ± 0.72	13.89 ± 1.12	21.38 ± 1.53	24.09 ± 1.20	29.42 ± 0.52
V_7	20.13 ± 1.03	22.07 ± 0.05	27.30 ± 0.24	30.52 ± 0.96	46.61 ± 1.76
V_8	10.35 ± 0.49	11.00 ± 0.48	13.70 ± 0.80	16.03 ± 1.26	22.10 ± 0.60
V_9	13.57 ± 0.63	16.96 ± 1.09	17.44 ± 0.23	17.62 ± 0.45	21.31 ± 0.05
V_{10}	13.77 ± 0.44	13.99 ± 0.70	14.26 ± 0.78	15.10 ± 1.37	21.97 ± 1.67
48HAT					
48HAT V_1	14.55 ± 0.22	17.97 ± 0.60	18.20 ± 1.17	22.27 ± 0.61	22.88 ± 1.05
V_2	21.97 ± 0.60	23.12 ± 0.91	24.89 ± 0.93	29.52 ± 0.81	44.07 ± 1.01
V_3	20.45 ± 0.61	30.49 ± 1.74	40.51 ± 0.17	46.55 ± 0.82	48.24 ± 1.42
V_4	26.64 ± 0.41	30.75 ± 0.60	36.52 ± 0.69	50.23 ± 1.30	54.84 ± 0.38
V_5	26.61 ± 0.42	40.20 ± 0.77	48.84 ± 1.48	60.86 ± 0.75	65.87 ± 0.97
V_6	14.76 ± 0.41	16.97 ± 2.12	29.56 ± 2.82	32.46 ± 1.09	32.35 ± 1.51
V_7	24.50 ± 0.87	23.25 ± 2.17	28.62 ± 1.71	41.65 ± 0.17	52.73 ± 2.46
V_8	16.99 ± 0.16	20.95 ± 0.66	24.51 ± 1.58	32.10 ± 0.38	34.87 ± 0.99
V_9	15.17 ± 0.98	19.07 ± 0.62	21.50 ± 0.52	23.96 ± 0.94	24.24 ± 0.87
V_{10}	14.68 ± 0.44	23.96 ± 1.01	23.13 ± 0.13	24.92 ± 0.88	22.98 ± 0.41
72HAT V_1	18.35 ± 0.97	20.83 ± 1.07	21.69 ± 0.91	23.14 ± 0.32	24.06 ± 1.02
V_2	21.77 ± 0.27	23.97 ± 1.89	28.29 ± 1.01	35.33 ± 1.47	45.26 ± 0.82
V_3	23.85 ± 0.45	44.01 ± 0.32	49.22 ± 1.58	52.34 ± 0.21	57.29 ± 1.71
V_4	26.35 ± 12.05	34.60 ± 0.60	42.17 ± 0.75	42.44 ± 0.35	57.74 ± 0.83
V_5	28.33 ± 0.83	47.35 ± 0.68	64.16 ± 1.34	68.76 ± 0.24	70.98 ± 1.49
V_6	17.37 ± 0.52	23.93 ± 1.61	29.13 ± 1.79	34.26 ± 0.47	37.65 ± 1.24
V_7	26.92 ± 0.32	34.55 ± 1.07	32.76 ± 0.75	55.36 ± 0.96	54.39 ± 1.03
V_8	19.26 ± 0.87	22.40 ± 0.60	28.28 ± 0.40	34.87 ± 1.82	36.60 ± 0.90
V_9	17.78 ± 0.46	23.06 ± 0.59	23.52 ± 0.67	25.75 ± 0.65	28.42 ± 0.20
V_{10}	18.81 ± 0.38	24.77 ± 0.86	25.05 ± 0.67	27.36 ± 0.52	29.33 ± 0.76
CD at 5%:		**24HAT**	**48HAT**	**72HAT**	
Genotypes*		**2.6552**	**2.9804**	**2.5867**	
Treatments*		**1.8775**	**2.1074**	**1.8291**	
Genotypes x Treatments*		**5.9373**	**6.6643**	**5.784**	

Table 8: Variations in ATP-sulphurylase activity (EU mg^{-1} protein min-1) of *B. juncea* L. genotypes under Cd-stress.

Genotypes	Treatment (mM CdCl2)				
24HAT	T_0	T_1	T_2	T_3	T_4
V_1	0.99 ± 0.01	1.02 ± 0.01	1.02 ± 0.01	1.10 ± 0.01	1.13 ± 0.12
V_2	1.35 ± 0.02	1.40 ± 0.01	1.46 ± 0.01	1.52 ± 0.01	1.61 ± 0.13
V_3	1.78 ± 0.03	1.84 ± 0.02	1.85 ± 0.02	1.91 ± 0.02	2.21 ± 0.05
V_4	1.65 ± 0.02	1.65 ± 0.05	1.67 ± 0.02	1.72 ± 0.02	1.75 ± 0.05
V_5	1.61 ± 0.01	1.67 ± 0.02	1.69 ± 0.01	1.75 ± 0.03	1.88 ± 0.05
V_6	1.57 ± 0.03	1.65 ± 0.02	1.66 ± 0.02	1.67 ± 0.03	1.68 ± 0.03
V_7	1.43 ± 0.02	1.43 ± 0.01	1.46 ± 0.02	1.53 ± 0.01	1.68 ± 0.03
V_8	1.54 ± 0.01	1.55 ± 0.02	1.58 ± 0.03	1.58 ± 0.03	1.65 ± 0.01
V_9	0.96 ± 0.02	0.99 ± 0.02	1.07 ± 0.12	1.11 ± 0.02	1.13 ± 0.02
V_{10}	1.21 ± 0.01	1.26 ± 0.03	1.29 ± 0.14	1.31 ± 0.06	1.38 ± 0.05
48HAT V_1	0.99 ± 0.02	1.03 ± 0.02	1.18 ± 0.02	1.15 ± 0.01	1.19 ± 0.01
V_2	1.36 ± 0.03	1.40 ± 0.03	1.47 ± 0.01	1.62 ± 0.02	1.70 ± 0.07
V_3	1.84 ± 0.01	1.91 ± 0.02	1.97 ± 0.02	2.12 ± 0.02	2.35 ± 0.05
V_4	1.67 ± 0.01	1.73 ± 0.02	1.81 ± 0.01	1.81 ± 0.05	1.86 ± 0.02
V_5	1.65 ± 0.01	1.72 ± 0.01	1.73 ± 0.00	1.77 ± 0.04	2.04 ± 0.02
V_6	1.61 ± 0.02	1.73 ± 0.02	1.77 ± 0.03	1.81 ± 0.04	1.89 ± 0.02
V_7	1.46 ± 0.02	1.48 ± 0.02	1.53 ± 0.04	1.67 ± 0.02	1.76 ± 0.03
V_8	1.58 ± 0.02	1.60 ± 0.01	1.67 ± 0.05	1.73 ± 0.03	1.87 ± 0.06
V_9	1.00 ± 0.03	1.07 ± 0.01	1.20 ± 0.05	1.23 ± 0.06	1.25 ± 0.01
V_{10}	1.26 ± 0.01	1.32 ± 0.03	1.39 ± 0.06	1.38 ± 0.02	1.41 ± 0.05
72HAT					
V_1	1.10 ± 0.001	1.16 ± 0.01	1.20 ± 0.005	1.30 ± 0.001	1.32 ± 0.01
V_2	1.41 ± 0.003	1.55 ± 0.01	1.59 ± 0.003	1.73 ± 0.01	1.82 ± 0.01
V_3	1.86 ± 0.003	1.96 ± 0.004	1.99 ± 0.01	2.19 ± 0.02	2.52 ± 0.01
V_4	1.75 ± 0.002	1.78 ± 0.003	1.85 ± 0.003	1.99 ± 0.002	2.25 ± 0.04
V_5	1.71 ± 0.003	1.76 ± 0.01	1.87 ± 0.003	1.88 ± 0.002	2.27 ± 0.02
V_6	1.62 ± 0.001	1.76 ± 0.003	1.87 ± 0.07	1.91 ± 0.02	1.95 ± 0.01
V_7	1.48 ± 0.003	1.55 ± 0.01	1.63 ± 0.01	1.85 ± 0.01	1.91 ± 0.01
V_8	1.60 ± 0.002	1.63 ± 0.003	1.76 ± 0.005	1.87 ± 0.002	1.88 ± 0.005
V_9	1.11 ± 0.003	1.17 ± 0.002	1.21 ± 0.004	1.28 ± 0.003	1.32 ± 0.002
V_{10}	1.31 ± 0.002	1.39 ± 0.003	1.47 ± 0.003	1.50 ± 0.01	1.55 ± 0.01
CD at 5%:		**24HAT**	**48HAT**	**72HAT**	
Genotypes*		**0.0511**	**0.218**	**0.0743**	
Treatments*		**0.0361**	**0.0154**	**0.0554**	
Genotypes x Treatments*		**NS (Non-sgnificant)**	**0.0486**	**0.1537**	

Discussion

The *Brassica juncea* genotypes countered extensively with reference to the biochemical parameters and differential tolerance to Cd-stress. Restriction of Cd- transport from root to shoot has been considered as a type of mechanism of plant tolerance to stress. Increased Cd supply resulted into the higher accumulation of this metal in roots. Additionally, more Cd was accumulated in root followed by shoot. The pattern of Cd distribution in various plant organs of the *Brassica juncea* L. genotypes as observed in this study, suggests reduced translocation of Cd from root to the shoot with increased Cd supply which is considered a means of tolerance mechanism [57]. A clear difference among genotypes in Cd accumulation was observed at 24 hrs of exposure of the seedlings to the Cd, it was further increased over the experimental period. The movement of Cd from root to shoots is a vital factor affecting accumulation of this metal in shoot system of Brassica juncea L. genotypes. The increasing concentrations of Cd in shoots over the experimental period might also be due to the disturbed root function to some extent, because of decreasing micronutrient concentra-

tions [58]. Another possibility might be that at higher concentrations the selectivity of plasma membrane is reduced, thus allowing more rapid entry of Cd. Besides Cd-translocation is not only governed by transporters but also through the water channel called aquaporin (AQPs). AQPs are the members of Major Intrinsic Protein family (MIP) with a molecular weight ranging from 23 to 31 kDa [59-61].

The results of our study also suggest that vacuolar compartmentalization may dominate in the genotype V5 (Pusa Jai Kisan), resulting in the accumulation of higher levels of Cd in this genotypes. Our results are in confirmation with several reports proposing that Cd accumulation and inactivation in the cells of root are most probably related to cell wall binding, vacuolar compartmentalization in complexed forms, and are the factors that decisively effect heavy metal hemostasis [62-66]. More Cd accumulation in V5 is supposed to be due to its ability to form complexes with various chelating agents, thus facilitating transport of additional Cd.

Plants retaliate to heavy metals quickly by way of accumulation of stress specific metabolites at cellular and sub cellular levels such as amino acids, imino acids, organic acids mostly succinic, malic and galacturonic acids which are required for their survival and exhibit great variations with species, genotypes and stress factor [68]. They also get adapted with different members of metal transporter families [69], chelators and metalloproteins [70], various hsps which are capable of building innate immunity system in plants [71] apart from modulation, transportation, accumulation, and correction of metal metabolic imbalance. Thus increase in protein level in the leaves may be because of their incorporation in specific metal binding proteins [72]. An increase in protein content on Cd exposure was noted [73], in spite of the decreased ATP supply from mitochondria and chloroplast for protein synthesis.

Expression of metallothionein (MT) genes have been found to enhance in several plants during senescence induced by abiotic and biotic stressors including heavy metals [74]. There are some Cd stress specific genes, necessary for the detoxification process whose expression results in an increased amount of specific proteins. A second and more important reason for the increase in protein content might be that the activity of acid proteases in present study does not undergo the rapid rise till 48 HAT, so little proteolysis does occur up to that time. The decrease in protein content as observed at 72 HAT may be because of the increased protein degradation process that was supported by increased protease activity. Membrane damage as a result of Cd stress allows mixing of proteases with other proteins resulting in protein loss. The chloroplast proteins are reported to disappear more rapidly than cytoplasmic proteins under stressful conditions. Perhaps the loss of these proteins during Cd stress is a plant response, utilizing these stored proteins for its survival under stressful environment. It has also been reportd that Cd exposure relocates RuBP carboxylase/oxygenase activity more towards its oxygenase function [75].

Cadmium stress alters the size and composition of the amino acid pool. The influence of Cd on amino acids may be divided in three groups: (a) amino acids that grows with Cd concentration, (b) amino acid that reduces and (c) amino acids that are unaffected. Cadmium influences the amino acid pool of the plant, because of dysfunction of water flux [76]. We observed a rise in soluble amino acid content in these *B. juncea* L. genotypes under Cd stress. This greater accumulation of amino acid content with Cd treatment may be due to the sored proteolysis of the cellular proteins or de nove synthesis of amino acid in Cd-treated plants. This might be correlated with the dip in protein content in *B. juncea* L. genotypes in our study at the time when amino acid levels were higher. Protein degradation and catabolism of amino acid represents the modification of plant cells to get through nutrient starvation a result of the decreased CO_2 assimilation rate through autophagic processes activated by Cd-induced oxidative stress [77]. This process is necessary for sustaining cellular homostasis [78]. Thus, aggregation of amino acids in Cd treated plants may be the result of one of several probabilities: (i) it might be as a result of reduced respiratory metabolism [79], due to disorganization of membranes [80], resulting into the accumulation of several cellular respiration -citric acid cycle compounds like 2-oxoglutarate that advocates the synthesis of specific amino acids, (ii) increase in the levels of specific amino acids, methionine and cysteine [81] as Cd activates the enzymes involved in their synthesis [82] asparagine required for N-metabolism [83] and (iii) a declined protein synthesis that donates to the accumulation of amino acids particularly at high concentration.

Proteases may be of different types. Some may exclusively breakdown native cellular proteins and peptides, while others may be effective in breakdown of extracellular proteins. Of the major aspects of Cd induced damages, which are as yet not at all clear, one specifically concerns the proteases. It is because of lack of close correlation between protease level and protein breakdown, encountered both in our own work as well as of others [84]. Our results showed an increase in proteolytic activity in the *Brassica juncea* L genotypes at all the studied stages. The impact of Cd-induced oxidative stress is the induction of numerous protease activities by a post-translational mechanism resulting in programmed cell death (PCD) or cellular suicide. PCD is accelerated through generation of ROS that get increased under Cd stress (Jiang et al., 2019). Smaller doses (deteriorating agents) induce antioxidant enzymes, however, when the concentration of ROS reaches a certain threshold, they act as a remarkable hazard that ultimately leads to PCD [85,35]. Also it has been suggested that Cd triggers the process of PCD by a channel that requires the endoplasmatic reticulum (ER), in which unfolded proteins accumulate [86], as was found in Nicotiana tabacum BY-2 cells in which markers for ER stress (Nt-BLP4 and NtPDI) were up regulated after exposure to Cd. In our results the low protease activity of V5 might be most probably related to low ROS generation [26]. The differences in the protease activity among *B. juncea* L. genotypes suggest that changes in proteolytic activities are not random events, but may have a degree of genotypic specificity [26] (Greenberg, 1994).

Cd is known to induce thiol based complexing substances, phytochelatins [88], through the up-regulation of glutathione biosynthesis [89], that mediates the detoxification process. The tripeptide glutathione is the most rich form of organic sulphur in plants apart from that assimilated in proteins. Plants use phytochelatins to chelate heavy metals on the thiol moiety of cys, resulting in safe complex formation. Among the responses of plants to Cd stress, the increases in the pool of GSH have been reported from many plant species. Increased level of GSH in *Brassica juncea* L genotypes under Cd stress [26], suggest its vigorous involvement in quick withdrawal of ROS directly (non-enzymatic) as well as enzymatically [88] (Asada and Takahashi, 1987). GSH is synthesized under pressure; to form phytochelatin and activates the sulfur uptake as well as its own production

as cysteine is the precursor for both [90-92].

The amount of non-protein thiols (NPTs) increased in a dose dependent manner in all the *B. juncea* L. genotypes with Cd stress. The traceable increase in thiol containing components in Cd treated cells results from de-novo synthesize [93], even though it is relocated towards the synthesize of PCs [90]. This mechanism is compulsory because PCs detoxificates metals owing not purely to their function as cytoplasmic metal chelators, but also because of its compartmentalization and stabilization in vacuole [15]. The escalated level of non-protein thiols under Cd stress is particularly evident as many steps in the biosynthetic pathway of thiol (cys) are induced by heavy metals [94]. The variability in the volume of thiols between *B. juncea* L. genotypes conceivably may be related to the difference in enzyme activities involved in sulphur assimilation. The increased amount of thiols under Cd toxicity is credited to increased sulphur uptake in wildtype sel 1–10 [90]. In V1, there was a slight increase in non-protein thiols. This might be due to the decreased EC-synthetase activity to make EC and thus keeps the cysteine and glutathione pool low and deprives PC formation. In V5, the activity of EC synthetase activity might be high resulting in an increased EC formation, eliciting that under same conditions EC synthesis is enhanced in unsusceptible plants more than its transformation to GSH. Further, the increased capacity for their formation in these genotypes with high EC-synthetase protein results in increased EC levels that result in high non-protein thiols as well as GSH content as compared to those found in sensitive plants. Phytochelatin synthesize is regarded as a biomarker for cellular Cd sequestration, because genetic analysis has confirmed the involvement of PCs in Cd detoxification [95,96]. PCs are synthesised in cytosol, where they have a high harmony for binding with heavy metals, particularly Cd, and are then transported into the vacuole thus sequestering the metals away from the sensitive enzymes [82]. This system provides a physiologically and biologically well-conserved procedure proposed to deal with metal toxicity [95]. The major detoxification mechanisms in plants are based on vacuolar compartmentalization [97], and ligand complexation. Phytochelatins are induced in plants after exposure to a number of heavy metals including Cd. Our results indicate an increased level of PCs under Cd exposure and the increase was more prominent over the Cd exposure time. V5 accumulated more PCs hence, more Cd resistance. Early and rapid formation of Cd-binding peptides (CdBP-Cd) has generally been found to correlate highly with metal tolerance of cultured cells [82]. The low PC content in V1 might be not only due to decreased GSH level [26]), but it might be limited also by the rate of PC synthase or the capacity for further processing and/or transport of Cd or Cd-phytochelatin complex. Comparison of clones of Cd-sensitive and Cd tolerant populations of Silene-vulgaris plants has also shown that more PCs are produced in tolerant clones than in sensitive genotypes and the PCs in the tolerant clones bind twice as much as Cd as those in sensitive clones. Clearly the evidence above suggests that Cd detoxification or tolerance in plants may be, at least in part, achieved by CdBP (cadmium-binding protein) induction and CdBP-Cd complex formation after metal exposure.

During senescence, the genes related to the metabolism of sulphur shows high expression. Brassica napus gene encoding ATP-sulphurylase shows enhanced expression during senescence. This enzyme activates sulphate in the presence of ATP, in the first step of cysteine and methionine biosynthetic pathway [98] (Schmidt and Jäger, 1992). Free cysteine is converted to the tripeptide glutathione and is known be under tight S-regulation pathways based on demand, supply and assimilation, playing an important role in response to abiotic stress including Cd. Increase in the cysteine concentration concomitant with an increased expression of the enzyme for sulphur assimilation, ATP-sulphurylase (AS) and adenylsulphate reductase (AR) have also been reported after Cd exposure in *Brassica juncea* L. genotypes. Our results showed an increase in ATP-sulphurylase activity in *Brassica juncea* L. upon treatment with cadmium. The increased ATP-sulfuralyase activity has also been reported for salt tolerant genotypes of *Brassica juncea*. [99]. The greater ATP-sulphurylase activity might be due to the de novo synthesis of PCs, which require an increased synthesis of tripeptide glutathione that in turn depends on increased sulphur assimilation [100]. Studies on Canola have shown that in response to sulphur starvation, the concentration of glutathione in phleom declines [101], and this may be the signal for observed upregulation of sulphate transporter and ATP-sulphurylase activities. The rate-limiting step in PC biosynthesis is provision of cys, and therefore the availability of reduced sulphur is of prime importance [102]. Upon heavy metal stress some genes involved in the sulphur assimilation pathway are known to be transcriptionally activated, resulting in an elevation of enzymatic activity [103]. Reverse genetic approaches have also revealed sulphur assimilation pathway involvement in Cd tolerance mechanism in *Brassica juncea* L. [104,105]. The present work suggests that cadmium may activate the sulphur assimilation, by increasing transcription of related genes to provide a greater supply of cysteine or glutathione for PC biosynthesis as was reported by [106]. Since the rate of AR activity increased significantly when plants that were briefly exposed to Cd were transferred to Cd free medium. Cadmium could directly inhibit AS and AR. In particular AR contains several essential cysteine residues [107], with which free Cd could form a complex, thereby inactivating the enzyme.

The results obtained in this study suggest that Cd stress resulted in differential ultrastructural changes in the chloroplasts and leaf mitochondria of the three *Brassica juncea* L. genotypes under study. The major responses elicited by the chloroplast to Cd stress are the deorganisation of outer membrane, thylakoid membrane and disappearance of granal stacks. Damage to the membrane structure of the chloroplasts and mitochondria under Cd treatment appears to be primarily because of the accumulation of toxic ions in these organelles. Cadmium accumulation in cellular organelles like nuclei, mitochondria and chloroplasts has also been reported in many plant species as a possible mechanism of plant for Cd compartmentalization. But this strategy does not serve as a defense mechanism, but may result in loss of integrity, genome instability and toxicity [108,109], causing dismantling of the cell's organellar complement. This is supported by many studies that show biotic and abiotic stress induce severe ultra structural changes in cellular and sub-cellular organelles, like invagination in nuclear membrane, spindle fiber abnormality (SFA) and chromosomal abnormality [110], degeneration of chloroplast, loss of starch, alterations in the size of plastoglobules, thylakoids and are considered authentic stress markers [111,112]. Similarly Cd decreases mitochondrial membrane potential, increases its disintegration, decreasing its electron transport which results in the enhancement of ROS production [113]. They plays a key role not only in photorespiration but in the metabolism of various amino acids, vitamins and lipids which are important for biomembrane synthesis and maintenance. Further, they have an intimate relationship with the chloroplast and the integration of redox signals, therefore considered as the important competi-

tors in metal-induced cellular response. Many researchers have also suggested an increase in the lipooxygenase activity during various abiotic stresses [114,115], and its role in the modification of biological membranes causes adverse structural changes in plants. Lipoxygenase catalyses polyunsaturated fatty acid oxidation and produces free radicals from these fatty acids that in turn cause the local destruction of the plastid membrane [116]. Among the genotypes studied for the said parameters, less damage was observed in V5. A little damage to thylakoid membranes and cristal organization of resistant genotypes also coincide with its better metal toxicity resistance, since they harvest pigments, enzymes and other molecules that take part in light reaction of photosynthesis, photorespiration, organic acid production and enzymes (glutathione-S-transferase -GST) responsible for stress detoxification. However further work is needed to explore the role of mitochondria in conferring resistance to plants as it functions as both a victim and adjustor of stress responses in plants. The sensitivity to and the response against Cd significantly differed among Brassica cultivars under study. This may be correlated to better antioxidant and metal sequestration defense mechanisms developed in tolerant variety that have protected it from the detrimental effect of Cd toxicity [117, 26]. Thus the findings presented here suggest that this variety is appopros both for Cd phytoremediation and biofortification.

Conclusion

Cadmium is well known toxic pollutant in the environment responsible for the malfunctioning of cellular systems. A comparative study was made among ten Brassica juncea L genotypes giving them exposure to Cd that elucidated varying susceptibility and stimulation of defense molecules. From the results of our study, it can be concluded that Cd-tolerant genotype of Brassica juncea (Pusa Jai Kisan) is equipped with well-developed mechanism that makes it a potential genotype to be used for phytoremediation process of heavy metals, specifically for the soils contaminated with Cd. PCs produced by *B. juncea* L. genotypes, although considered to be highly efficient for Cd detoxification, cannot be taken as the only available mechanism to fight Cd stress. Our study suggest that exposure to Cd implicit an amalgamated response, involving not only PCs, but also subsequent mechanisms of stabilization and compartmentalization of the ligand-metal complex and the production of non-protein thiols, that strengths cellular antiotoxicant system. Further experiments are necessary to directly demonstrate and understand their participation in detoxification of Cd. Better results may be obtained by working out with mutants for biochemical and physiological analysis as well as elucidating the nature of the gene for differential metal tolerance. That will definitely help to optimize the process of phytoremediation. Further, screening for more hyper tolerant and hyper accumulator genotypes could be rewarding.

Conflict of interest: All the authors hereby declare that they have no conflict of Interest.

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