Physiological and Biochemical Changes to Low Doses of Cadmium in Two Varieties of *Oryza sativa* L. of Odisha, India

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Abstract: ***Cadmium (Cd) is considered to be a non-essential heavy metal which have a significant impact on plants. Effect of cadmium on physiological as well as biochemical activities of two rice plants (var.– Udaygiri and var. Jagabandhu) were studied. The germination percentage of var. Udaygiri was 88.89 % at concentration 20 µM, 26.67% in 400 µM and that of var. Jagabandhu was 85.55 % in 20 µM, 21.11 % in 400 µM after four days of Cd treatment. The total chlorophyll content of var. Udaygiri and var. Jagabandhu at 400 µM was 248.44µg/g , 167.75µg/g respectively. Carotenoid content of var. Udaygiri and var. Jagabandhu at 400 µM was 98.93µg/g , 103.89 µg/g respectively. Starch content for both var. Udaygiri and var. Jagabandhu was found to be 14.84µg/g, 9.68µg/g respectively at 400 µM. The total sugar content at 400 µM was 3.01 µg/g and 2.26 µg/g for var. Udaygiri and var. Jagabandhu respectively. The proline content increased upon exposure to 20 µM to 400 µM cadmium. The above result revealed that increased Cd concentration in the medium showed decreased germination rate, photosynthetic activity, total sugar, starch, but there was an increase in the proline content at different time intervals (7-21 days).***

Keyword : Cadmium, carotenoid, chlorophyll, heavy metal, proline

1. Introduction

Heavy metals are known to compete with other minerals that serve as essential nutrients for plants and therefore are thought to disturb the nutritional value of plants [1]. They get absorbed by the roots of the plants and therefore get accumulated in various tissues as well as cell compartments of the plant and hence disturb the metabolic system of the heavy metal exposed plant [2,3,4]. Cadmium is a trace element which is known to have no significant biological functions, but is considered as highly toxic for living organisms [5]. As compared to other heavy metals it is known to be one of the most dangerous and harmful pollutant, and large amount of it is discharged into the environment mostly by human activities [6]. It tends to enter the environment and disturb the biogeochemical cycle and gets accumulated in soil and sediments. The roots of the plant absorb Cd and then Cd gets transported into the stem and leaf tissues [7]. Cd gets readily absorbed and translocated at a faster rate in plant system and therefore exerts very strong toxic effect even at relatively low concentrations [8]. Cadmium salts are used to control fungal infections [9,10]**.** Cd is phytotoxic, at high doses it results in plant growth retardation and creates obstacle in plant growth and development [11]**.** Cadmium is known to efficiently inhibit photosynthesis [12,13,14]**.** It tends to affect stomatal opening in higher plants [15] and causes an overall growth retardation, decreased photosynthetic pigments and other physiological processes [13,16,17].

Rice is considered to be an important field-crop throughout the globe and is known to be a model plant among monocots for scientific research due to its small genome size [18,19,20]. Cd contaminated rice grains causes serious health risk and affect half of the world's population, as it is one of the important staple food. It causes toxicity in rice plant and causes retardation of growth and inhibition of chlorophyll biosynthesis [21]. Seed germination is an important and generally thought to be a complex physiological process, which is very much sensitive to heavy metal pollution. Cd retards germination rate and negatively affects growth of seedlings [22,23]. The present paper discusses the toxic symptoms induced by Cd on various physiological as well as biochemical processes such as germination percentage, photosynthetic activity, total sugar, starch and proline content of two rice varieties i.e. var. Udaygiri and var. Jagabandhu .

2. Materials and Methods

2.1. Plant Material Collection

Rice seeds (*Oryza sativa* L.) were collected from the Plant Genetics and Breeding department, Orissa University of Agriculture and Technology (OUAT) , Bhubaneswar, Odisha and used as primary explants. Two rice cultivars (var. Udaygiri and var. Jagabandhu) were used for all experiments in this study.

2.2. Seed Treatment and Germination Assay

Rice seeds were taken and surface sterilized with 70% ethanol for 5 min followed by treatment with 0.01% $HgCl_2$ for 10 min and then washed with sterilised distilled water three times [10]. Thirty sterilised seeds were kept on filter paper containing 10ml of cadmium chloride monohydrate ($CdCl_2.H_2O$) solution of different concentrations (20 µM, 50 µM, 100 µM, 200 µM, 400µM). Controls were kept on filter paper soaked with 10 ml distilled water. The seeds were kept for four days under dark at controlled condition in culture room. In this study, germination was considered when the radicals were longer than 2 mm. Three replicates were taken for each treatment, each one consisting of 30 seeds. The radicle length was measured after 4 days of germination. The seedling vigour index [24], metal tolerance index [25] and phytotoxicity percent were calculated. The experiments were repeated three times with three replicates for each Cd concentration.

2.3. Extraction and Estimation of Photosynthetic Pigments

0.5 gm fresh leaves were taken and homogenized in chilled 80% acetone with the help of a morter and pestle. The homogenates were centrifuged at 10,000 rpm for 10 min in the dark [26] . The supernatant were collected and absorbance at 470nm, 646.8nm and 663.2nm were taken using UV –Visible double beam spectrophotometer (Perklin Elmer, Lambda 25, India) .The experiments were repeated three times with three replicates for each treatment at different Cd concentrations.

2.4. Extraction and Estimation of Total Sugar

Fresh leaves (0.1gm) were chopped and kept in a test tube with 5ml of 2.5N HCl and boiled in water bath for about 1 hr for hydrolysis. The sample was then neutralized by adding solid sodium carbonate till effervescence ceases. The volume was made up to 10 ml and centrifuged. The supernatant was collected and 0.5ml of the aliquots were taken for analysis. The volume was made up to 1ml by adding distilled water. Anthrone reagent (4ml) was added, heated for 8 min in boiling water bath. Cooled rapidly and absorbance was taken at 630nm as described earlier [27]. The experiments were repeated three times with three replicates for each concentration of Cd.

2.5. Extraction and estimation of starch

Fresh leaves (0.1gm) were homogenized in hot 80% ethanol by using a mortar and pestle. The homogenate was centrifuged to retain the residue. The residue was repeatedly washed with hot 80% ethanol. The supernatant was discarded and the residue was dried well over a water bath. 5ml distilled water and 6.5 ml 52% perchloric acid were added and centrifuged for 20 min and the supernatant was collected. 4ml of anthrone reagent was added and the tubes were heated for 8 min in a boiling water bath. Cooled rapidly and the absorbance was taken at 630 nm [27,28]. The experiments were repeated three times with three replicates for each treatment of different Cd concentrations.

2.6 Extraction and estimation of proline

Proline content was estimated following the methods of Bates et al. (1973)[29]. 0.5gm fresh leaves were taken and homogenized with sulfosalicylic acid and filtered through Whatman No-2 filter paper. 2ml of the filterate was taken to which 2ml ninhydrin and 2ml glacialacetic acid was added. The mixture was then incubated at 100°C for 1h. The reaction was stopped rapidly by keeping the test tubes in ice chamber. 4ml toluene was added and the mixture was shaken vigorously for 15 - 20 sec. The aqueous toluene layer was separated and warmed to room temperature. The red color aliquote was measured at absorbance 520nm. The experiments were repeated three times with three replicates for different concentrations of Cd treatment.

3. Results and Discussion

3.1. Toxicity of cadmium on germination rate and radical length

The rate of germination rice seeds responded differently to different doses of cadmium concentrations. It decreased with increasing concentration of cadmium and extremely reduced when the concentration reached about 400µM as compared to 20µM (Table. 1 and Fig. 1). The germination percentage was 88.89% (20µM), 26.67% (400 µM) for var. Udaygiri and 85.55% (20µM), 21.11% (400 µM) for var. Jagabandhu. The radicle length of var. Udaygiri decreased with increasing cadmium concentrations 6.8cm (20µM), 2.4 cm (400 µM). Similar trend was seen in the case of var. Jagabandhu 5.06cm (20µM), 1.2cm (400µM). Seedling vigour index of var. Udaygiri was 604.45 and 64.008 for 20µM and 400µM respectively and that of var. Jagabandhu was 432.88 (20 µM) and 25.33 (400 µM). Metal tolerance index was recorded as 94.44 and 33.33 for 20µM and 400µM respectively in case of var. Udaygiri, 76.38 and 18.09 for 20µM and 400µM respectively for var. Jagabandhu. Phytotoxicity percent for var. Udaygiri was 5.56% and 66.67 % at 20µM and 400µM respectively and for var. Jagabandhu was 23.68% and 81.9% at 20µM and 400µM respectively.

Table 1: Effect of cadmium on seed germination of *Oryza sativa* L. (var. Udaygiri amd var.Jagabandhu)

Variety	Treatment	Germination percentage	Radical length (cm)	Seedling vigour index	Metal tolerance index	Phytotoxic %
Udaygiri	Control	98.88 ± 0.417	7.2 ± 0.294	711.936	100	0
	20µM	88.89 ± 0 .471	6.8 ± 0.262	604.45	94.44	5.56
	50µM	82.22 ± 0.471	6.2 ± 0.294	509.764	86.11	13.89
	100µM	67.78 ± 0.471	4.8 ± 0.245	325.344	66.67	33.33
	200µM	44.44 ± 0.942	2.9 ± 0.287	128.876	40.27	59.72
	400µM	26.67 ±0.816	2.4 ± 1.22	64.008	33.33	66.67
Jagabandhu	Control	94.44± 0.942	6.63 ± 0.188	626.137	100	0
	20µM	85.55 ± 0.942	5.06 ± 0.205	432.883	76.38	23.68
	50µM	75.55 ± 1.699	4.93 ± 0.329	372.713	74.37	25.64
	100µM	57.78 ± 1.699	3.83 ± 0.249	221.49	57.789	42.23
	200µM	34.44 ± 0.942	2.33 ± 0.205	80.36	35.175	64.86
	400µM	21.11 ± 0.942	1.2 ± 0.648	25.332	18.09	81.9

*Values in the table are mean ± SD of 3 replicates.

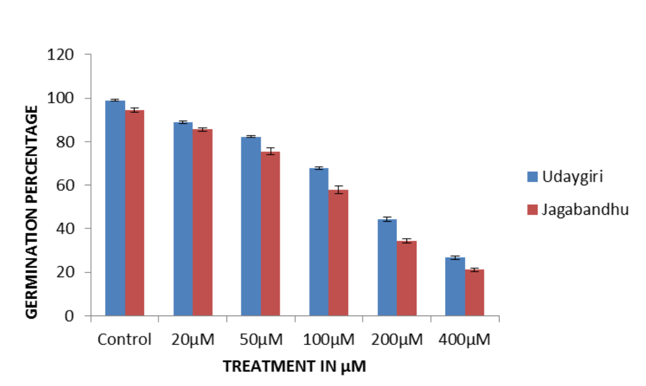

Figure 1: Effect of cadmium on seed germination of *Oryza sativa* L. (var. Udaygiri and var. Jagabandhu)

The seedling vigour index and metal tolerance index decreased with increase in Cd concentrations in the medium whereas the phytotoxicity percentage increased with increasing Cd toxicity. Hence, from all the above results we can clearly conclude that cadmium has a negative effect on the germination rate and growth of rice seedlings when exposed to increasing Cd toxicity.

3.2. Effect of cadmium on chlorophyll and carotenoid content

The change in photosynthetic pigments of var. Udaygiri and var. Jagabandhu is shown in the Tables 2a and 2b. A significant decrease in total chlorophyll was observed with increasing Cd concentrations. The carotenoids decreased with increasing days of exposure to different doses of Cd toxicity. The total chlorophyll content of var. Udaygiri at 21 days was 832.27 µg/g and 248.44 µg/g at 20 µM and 400µM respectively. Similarly total chorophyll content of var. Jagabandhu exposed to 21 days was 921.29 µg/g and 167.75 µg/g at 20 µM and 400µM respectively. Similar trend was seen in case of carotenoids. The carotenoid content of var. Udaygiri at 21 days interval was 225.37 µg/g and 98.93 µg/g for 20 µM and 400µM respectively. Carotenoids of var. Jagabandhu was 243.89 µg/g and 103.89 µg/g at 20 µM and 400µM respectively. The increased cadmium toxicity is thought to have disrupted the chloroplasts as a result of which there was a reduction and retardation of the photosynthetic pigments [30] of the two rice varieties when exposed to increasing Cd concentrations at different days of treatment.

Table 2a : Effect of Cadmium on total chlorophyll content of *Oryza sativa* L. (var. Udaygiri and var. Jagabandhu)

Variety	Treatment	Total chlorophyll (µg/g) f.wt.		
		Days of treatment		
		7	15	21
Udaygiri	Control	1178.03±4.5	1276.8±7.29	1323.92±31.08
	20 µM	975.34±5.18	935.63±16.67	832.27±4.51
	50 µM	889.15±1.31	768.64±8.02	669.85±17.43
	100 µM	585.05±5.72	416.41±20.13	314.09±17.4
	200 µM	425.2±11.87	295.83±10.46	256.98±1.08
	400 µM	361.57±0.99	287.11±0.34	248.44±8.71
Jagabandhu	Control	1140.01±0.75	1146.33±18.02	1173.25±27.63
	20 µM	1002.85±2.31	961.93±11.31	921.29±4.61
	50 µM	885.61±13.07	719.54±1.91	683.71±7.92
	100 µM	530.14±1.57	385.81±6	338.78±3.7
	200 µM	403.12±2.04	295.89±16.22	197.15±1.41
	400 µM	319.32±0.54	234.09±3.21	167.75±12.28

*Values in the table are mean ± SD of 3 replicates.

Table 2b : Effect of cadmium on carotenoid content of *Oryza sativa* L. (var. Udaygiri and var.Jagabandhu)

Variety	**Treatment**	**Carotenoid (µg/g) f.wt.**		
		Days of treatment		
		7	**15**	**21**
Udaygiri	Control	279.28±1.42	302.06±10.96	310..94±10.59
	20 µM	242.84±0.56	246.45±8.66	225.37±4.13
	50 µM	238.18±2.41	215.7±0.75	197.97±6.28
	100 µM	173.44±2.93	154.84±2.51	139.33±2.65
	200 µM	156.2±2.69	132.17±0.83	115.13±1.55
	400 µM	117.61±0.6	95.88±0.6	98.93±0.41
Jagabandhu	Control	273.32±3.89	276±0.21	278.22±6.06
	20 µM	251.01±0.94	250.51±0.31	243.89±4.05
	50 µM	228.91±4.45	199.26±0.34	193.93±3.06
	100 µM	206.37±3.84	186.47±0.31	179.92±0.9
	200 µM	194.55±0.17	161.32±1.19	145.09±2.87
	400 µM	137.14±0.95	126.1±1.22	103.89±3.59

*Values in the table are mean ± SD of 3 replicates

3.2. Effect of cadmium on sugar content

The total sugar content of var. Udaygiri at 21 days interval was 7.758 µg/g and 3.018 µg/g for 20µM and 400µM respectively. Similarly for var. Jagabandhu at 21 days the total sugar content was 6.51 µg/g and 2.26 µg/g respectively. The total sugar content of the rice varieties decreased with increasing concentrations of cadmium whereas the control rice varieties showed increase in total sugar content (Table. 3 and Figs. 2a and 2b). The total sugar content of the plants depends on photosynthetic activity of that plant. As the photosynthetic activity was retared by cadmium contamination and was not effecient so we can note that the total sugar also reduced with increasing Cd toxicity exposed at different days interval.

Table 3 : Effect of cadmium on total sugar content of *Oryza sativa* L. (var. Udaygiri and var. Jagabandhu)

Variety	Treatment	Total sugar in µg/g f.wt.		
		Days of treatment		
		7	15	21
Udaygiri	Control	13.13 ± 0.0135	13.445 ± 0.062	14.906 ± 0.047
	20 µM	9.27 ± 0.002	7.785 ± 0.004	7.758 ± 0.0015
	50 µM	7.787 ± 0.0045	7.691 ± 0.002	7.007 ± 0.007
	100 µM	6.802 ± 0.001	6.01 ± 0.0185	5.22 ± 0.002
	200 µM	5.399 ± 0.0025	4.34 ± 0.0105	4.11 ± 0.0295
	400 µM	5.998 ± 0.011	4.069 ± 0.0155	3.018 ± 0.0035
Jagabandhu	Control	10.77 ± 0.002	12.38 ± 0.01	13.866 ± 0.007
	20 µM	8.22 ± 0.057	7.72 ± 0.002	6.51 ± 0.051
	50 µM	7.73 ± 0.003	6.66 ± 0.099	4.52 ± 0.003
	100 µM	6.71 ± 0.001	5.23 ± 0.024	4.04 ± 0.03
	200 µM	4.74 ± 0.002	3.86 ± 0.003	2.67 ± 0.048
	400 µM	4.38 ± 0.006	3.31 ± 0.007	2.26 ± 0.002

*Values in the table are mean ± SD of 3 replicates

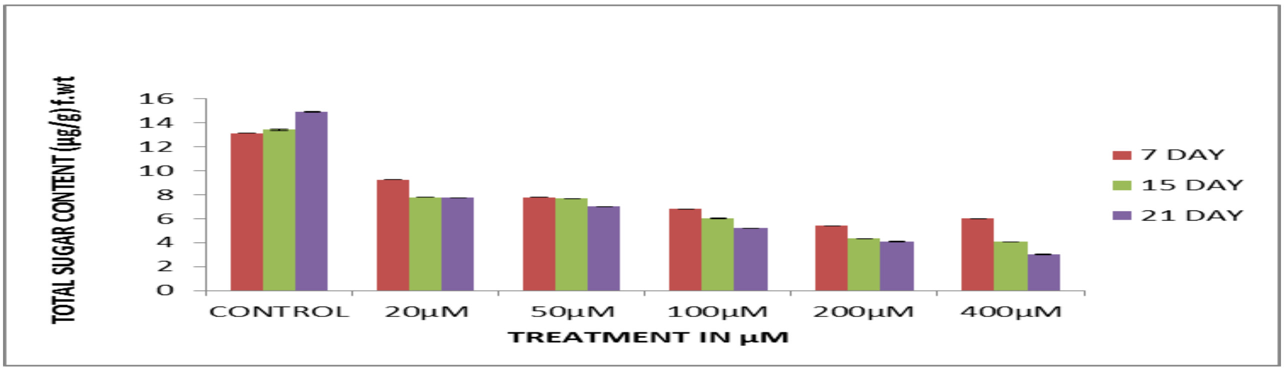

Figure 2a: Effect of cadmium on total sugar of *Oryza sativa* L. (var. Udaygiri)

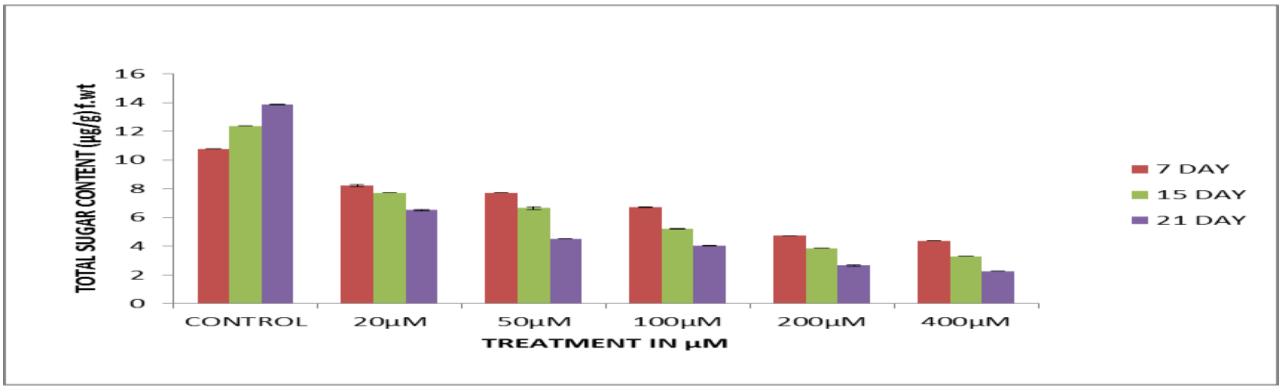

Figure 2b: Effect of cadmium on total sugar of *Oryza sativa* L.(var. Jagabandhu)

3.3. Effect of cadmium on starch content

The starch content of var. Udaygiri at 21 days interval was 46.791 µg/g and 14.846 µg/g for 20µM and 400µM respectively. Similarly for var. Jagabandhu at 21 days the total sugar content was 46.45 µg/g and 9.68 µg/g for 20µM and 400µM respectively. The starch content of the rice varieties decreased with increasing cadmium stress whereas the control rice varieties showed increase in starch content with incresing days (Table. 4 and Figs. 3a and 3b).

Table 4 : Effect of cadmium on starch content of *Oryza sativa* L. (var. Udaygiri and var. Jagabandhu)

Variety	Treatment	Starch content in µg/g f.wt.		
		Days of treatment		
		7	15	21
Udaygiri	Control	65.45 ± 0.0055	69.175 ± 0.0045	74.93 ± 0.0045
	20 µM	57.523 ± 0.008	53.169 ± 0.001	46.791 ± 0.0005
	50 µM	54.329 ± 0.0035	51.519 ±0.004	45.439 ± 0.032
	100 µM	48.105 ± 0.011	42.179 ± 0.002	38.284 ± 0.005
	200 µM	35.812 ± 0.06	33.586 ± 0.02	28.79 ± 0.002
	400 µM	23.861 ± 0.051	20.188 ± 0.0495	14.846 ± 0.027
Jagabandhu	Control	60.69 ± 0.047	66.64 ± 0.026	72.69 ± 0.011
	20 µM	57.41 ± 0.004	52.38 ± 0.04	46.45 ± 0.021
	50 µM	50.51 ± 0.0015	44.47 ±0.0005	39.43 ± 0.051
	100 µM	39.36 ± 0.051	35.96 ± 0.004	31.99 ± 0.033
	200 µM	32.031± 0.001	28.57 ±0.0175	23.46 ± 0.002
	400 µM	19.16 ±0.002	14.32 ±0.024	9.68 ± 0.006

*values in the table are mean ± SD of 3 replicates

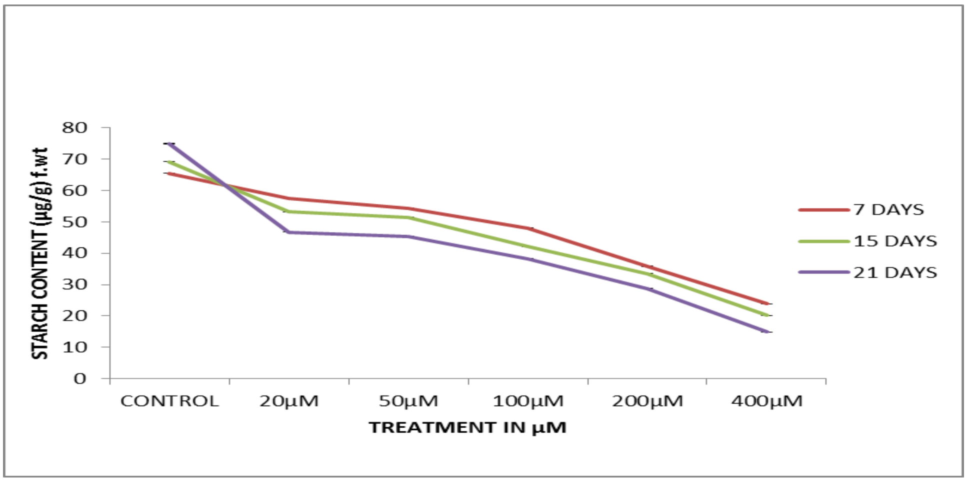

Figure 3a: Effect of cadmium on starch content of *Oryza sativa* L.(var. Udaygiri)

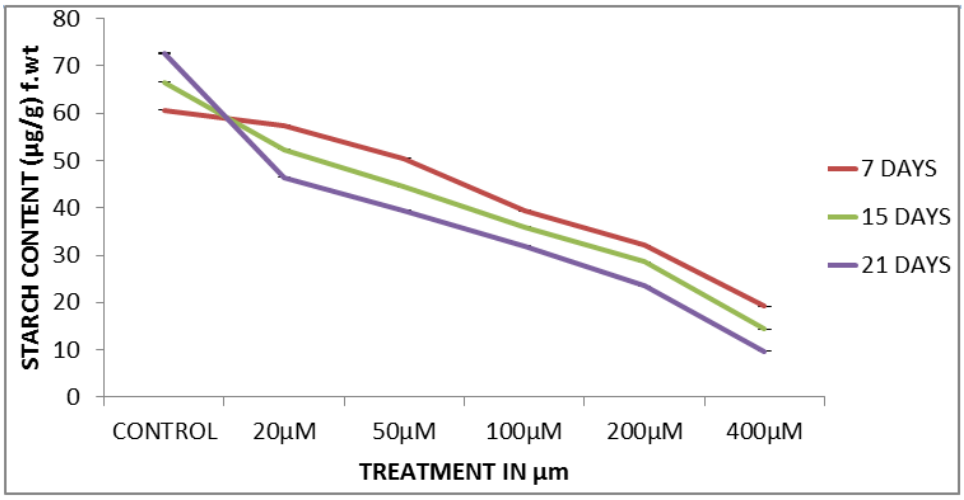

Figure 3b: Effect of cadmium on starch content of *Oryza sativa* L.(var. Jagabandhu)

3.4. Effect of cadmium on proline content

The Proline content of var. Udaygiri at 21 days interval was 4 µg/g and 70.6 µg/g for 20µM and 400µM respectively. Similarly for var. Jagabandhu at 21 days the proline content was 32.16 µg/g and 90 µg/g respectively. The proline content of the rice plants increased with increasing concentrations of cadmium whereas the control rice varieties showed slight increase in proline content but remained quite less that the cadmium treated plants. Proline content of the two varieties is shown in the Table. 5 and Figures 4a and 4b. Proline accumulation is a general phenomenon for all the stressed plants. As the rice plants were subjected to different cadmium stress, therefore estimation of proline was very much important to study whether increase in proline content can be a protection mechanism.

Table 5 : Effect of cadmium on proline content of *Oryza sativa* L. (var. Udaygiri and var. Jagabandhu)

Variety	Treatment	Proline content µg/g f.wt.		
		Days of treatment		
		7	15	21
Udaygiri	Control	1.95 ± 0.0004	2.67 ± 0.0009	2.86 ± 0.0041
	20 µM	2.01 ± 0.0028	3.31 ± 0.0037	4 ± 0.0046
	50 µM	13.35 ± 0.0016	17.98 ± 0.0045	19.19 ± 0.0216
	100 µM	25.1 ± 0.0195	40.73 ± 0.0245	44.91 ± 0.0036
	200 µM	37.32 ± 0.0081	47.17 ± 0.012	64.26 ± 0.0171
	400 µM	50.19 ± 0.0144	68.06 ± 0.022	70.6 ± 0.035
Jagabandhu	Control	12.74 ± 0.0037	17.89 ± 0.0094	30.85 ± 0.0009
	20 µM	15.67 ± 0.063	21.32 ± 0.0023	32.16 ± 0.0016
	50 µM	16.56 ± 0.509	30.91 ± 0.0009	41.26 ± 0.0023
	100 µM	22.72 ± 0.0023	37.29 ± 0.0117	55.75 ± 0.0014
	200 µM	38.07 ± 0.05	62.39 ± 0.285	76.75 ± 0.0009
	400 µM	52.75 ± 0.174	78.42 ± 0.0419	90 ± 0.0103

*values in the table are mean ± SD of 3 replicates

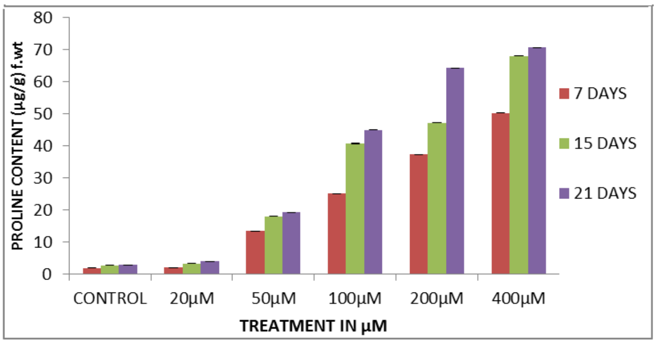

Figure 4a: Effect of cadmium on proline content of *Oryza sativa* L. (var. Udaygiri)

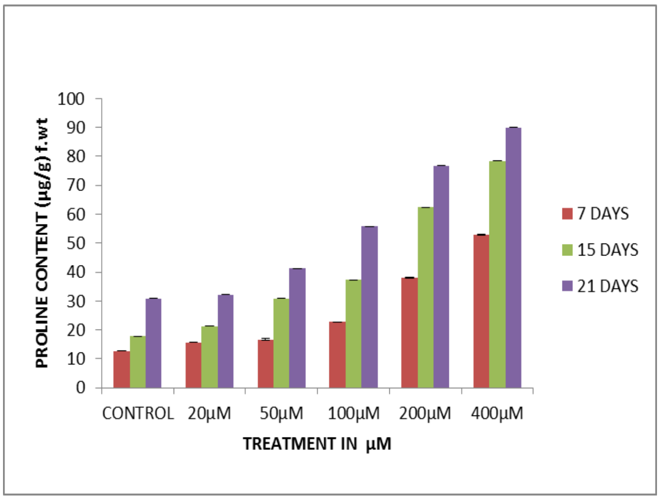

Figure 4b: Effect of cadmium on proline content of *Oryza sativa* L. (var. Jagabandhu)

4. Conclusion

Cadmium at relatively higher concentrations may inhibit the growth of the plant by directly inhibiting the germination, root growth. Cadmium therefore can inhibit the uptake of water and other essential mineral in plants, resulting in the uptake of cadmium itself and causing several essential mineral deficiencies. At high cadmium concentrations, the plants showed decrease in biochemical as well as physiological activities. Similar results were seen on the effect of cadmium [31,32,33,34,35]. Results from the germination studies indicated that var. Udaygiri showed somewhat higher resistance to cadmium as compared to var. Jagabandhu. This study also showed that at higher cadmium stress there is a significant decrease in photosynthesis, total sugar content, starch content whereas proline accumulation increased with increase in cadmium concentrations. As compared to var. Jagabandhu, var. Udaygiri showed good resistance effect. This study may help for selection of resistant variety for carrying out further research in the field of biology.

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