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Cadmium modulated defense of rice plant against fungal blast (*Pyricularia oryzae* Cav.) as evident by morphological and physiological changes

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ABSTRACT

Cadmium at lower concentration induces resistance to rice plants against *Pyricularia oryzae.* Seeds of *Oryza sativa* var. Khandagiri were tested in hydroponics treated with 50μM of $CdCl_2$, against control and infected with *Pyricularia oryzae* (casual organism of rice leaf blast). Morphological and physiological changes were monitored in 7d, 15d and 21d intervals after treatment with fungus. Significant decrease of plant height, chlorophyll pigment, carotenoids and increase of proline content were noticed in fungal treated plants that became normalized near control in co-stress plants. It was evident from the experiments that lower dose application of Cd provides resistant to rice against fungal pathogen.

1. Introduction

Rice (*Oryza sativa* L.) provides the staple food for more than half of the world's population (Sasaki and Burr 2000). Several biotic and abiotic stresses affect the plant growth and productivity among which are heavy metals and plant pathogens. Cadmium is phytotoxic at high doses which inhibits photosynthetic activity, retards the overall growth and physiological processes (Sheoran *et al*. 1990, Krupa *et al*. 1993, Chugh and Sawhney 1999, Jali *et al*. 2014). However, its yield and quality are also seriously compromised by infectious diseases caused by many pathogens. The rice blast fungus, *Pyricularia oryzae* Cav., is the most serious and destructive pathogen of rice plant. Mittra *et al*. (2004) reported low doses of cadmium provides resistant against fungal disease in wheat. Cadmium salts are also used against fungal infections (Osbourn 1996, Mittra *et al*. 2004). Use of fungicide to control the disease is ban in most of the developed countries due its accumulation as residual deposits in plant parts and various side effects on human health. Hence, an attempt was made to reduce the fungal incidence in rice plants by applying a micro dose of cadmium for developing systemic resistance.

2. Materials and methods

2.1 *Plant material collection and seed treatment*

Rice seeds (*Oryza sativa* L.) were collected from the Plant Genetics and Breeding Department, Orissa University of Agriculture and Technology (OUAT), Bhubaneswar, Odisha. *Oryza sativa var.* Khandagiri was used for all experiments in this study. Seeds were treated with 0.1% bevistine for 15 min., then washed three times with distilled water and kept in 70% alcohol for 30 sec and washed off with sterilized distilled water followed by treatment with 0.1% mercuric chloride for 5 min (Mittra *et al*. 2004). Seeds were finally washed three times with sterile distilled water and processed for seed germination.

2.2 *Collection and storage of Pyricularia oryzae*

The freeze-dried fungus (*Pyricularia oryzae* Cav.) culture was obtained from Institute of Microbial Technology (IMTECH), Chandigarh (India), bearing MTCC NO. 1477. Fungal spore suspension were routinely maintained on Oat meal Agar (OMA) slant and was inoculated into flask containing 100 ml Oat meal broth (Fig.1) and kept under

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aerobic condition in the dark at 25±1°C for 7 d in an incubator.

2.3 Hydroponic culture

Healthy germinated seeds were selected to be grown in thermocol cups containing Hoagland's nutrient solution and treated further with Cd^{2+} and *P. oryzae*. Seedlings grown in only nutrient solution without any other treatment served as control. The hydroponics were kept in culture room at 27°C temperature and 90% Relative Humidity and adequate light condition for proper growth.

2.4 Cd treatment and fungal infection

Seven days old seedlings were treated with 50 µM Cd. After 48 h the plant leaves were pricked with sterilized needle and 1ml (1×10^5 spores/ml) of *P. oryzae* broth culture was sprayed over the leaves of rice plant.

2.5 Antifungal activity

OMA media (19 ml) along with 1 ml of $CdCl_2$ was poured into petriplates and allowed to solidify. After complete solidification of the medium, fourteen day old culture of the *P. oryzae* was inoculated over the center of the media. The petri dishes containing OMA media devoid of $CdCl_2$ served as control. The plates were incubated at 25±1°C for seven days. After incubation the colony diameter was measured in mm (Singh and Tripathi 1999). Experiment was carried out in triplicates. The toxicity of $CdCl_2$ in terms of percentage inhibition of mycelial growth was calculated using the formula :

Percent inhibition = $C - T / C \times 100$, where C = Average increase in mycelial growth in control plate and T = Average increase in mycelial growth in treatment plate.

2.6 Extraction and estimation of photosynthetic pigments

Fresh leaves (0.5 gm) were thoroughly homogenized in chilled 80% acetone in a mortar and pestle. The homogenates were centrifuged at 10,000 rpm for 10 min at 4°C. The supernatant were collected and absorbance at 470 nm, 646.8 nm and 663.2 nm were recorded using UV–Visible double beam spectrophotometer for estimation of chlorophyll a, chlorophyll b, total chlorophyll and carotenoids (Arnon 1949).

2.7 Extraction and estimation of proline

Proline content was estimated following the methods of Bates *et al*. (1973). 0.5gm fresh leaves were homogenized with sulfosalicylic acid and filtered through Whatman No. 2 filter paper. 2ml of the filterate was mixed with 2ml ninhydrin and 2ml glacial acetic acid; the mixture was then incubated at 100°C for 1h. The reaction was stopped quickly 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 was measured at absorbance 520 nm. The experiments were repeated three times with three replicates.

3. Results and discussion

3.1 Germination and plant growth

The seeds found about 89% of germination. In control the rice plant, growth was very good which did not show any symptoms of chlorophyll deficiency and decreased plant height but the *P. oryzae* and Cd treated plants showed chlorophyll deficiency symptoms and reduced plant growth. No such chlorophyll deficiency was observed in co-stress (Fig.2).

3.2 Antifungal activity

Cadmium (50µM) treatment showed inhibition of fungal growth with increasing days of incubation whereas in control the growth increased with increasing days of incubation period. (Fig-3), (Table -1).

3.3 Photosynthetic pigments

There was a significant decrease of chlorophyll b except for control plants . Total chlorophyll and carotenoid contents decreased in pathogen treated plant as compared to Cd treatment which is at par with 21d control and Cd+Pathogen treated seedlings. The total chlorophyll and carotenoid contents in 21 d treatment were 428.29 µg/g in Cd treated plant and 325 µg/g in fungus treated plant and

Table 1

Percent mycelia inhibition

Days of treatment	Control (mm)	50 µM Cadmium (mm)	% Inhibition
7	15 ± 0.81	10.33 ± 0.47	31.11
15	18 ± 1.63	11.33 ± 0.94	37.03
21	25.33 ± 0.94	12 ± 1.63	52.63

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

145.36µg/g and 63.51 µg/g respectively. The carotenoid was 173.67 µg/g for co-culture which is significantly higher than single treatments. The co-culture showed increased chlorophyll as compared to single treatments but was lower than control. Thus, the fungus and cadmium treated plants did not showed enhance chlorophyll and carotenoid content, but in co-stress the photosynthetic pigment content increased significantly (Table 2).

3.4 Proline content

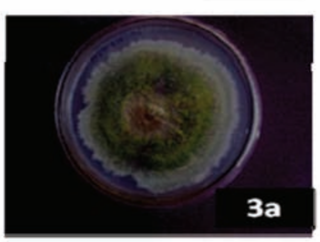
The proline content found 7.19µg/g to 10.71µg/g in control plants (7d to 21d). In fungus treatment, the proline content was increased significantly up to 29.78µg/g which was found less as compared to Cd treated plants (27.03 µg/g f.w.). In co-culture, after 21 d proline content decreased up to 10.095 µg/g (Fig. 4). Enhanced level of proline was reported to reduce oxidative stress damage to cells in rice. Thus, the co-stress infected plants have synergistic effect of PR protein along with Cd treated plant. Further it was observed that phytoallexin like substance due to fungal infection reduces production of proline concentration in accordance with accumulation of proline which is a general phenomenon in all the stressed plants (Lee *et al.,* 2003).

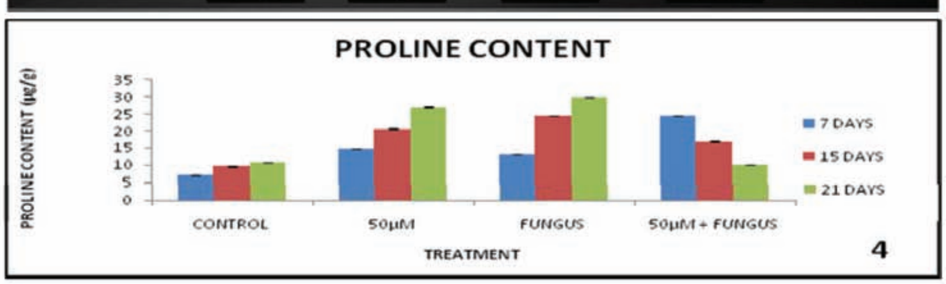
Table 2

Effect on photosynthetic pigments of rice.

Treatment	*Chl a* (µg/g f.w.) ± SD			*Chl b* (µg/g f.w.) ± SD			Total*Chl*(µg/g f.w.) ± SD			Carotenoid (µg/g f.w.) ± SD		
	7d	15d	21d	7d	15d	21d	7d	15d	21d	7d	15d	21d
Control	355.9± 8.8	365.9± 9.7	373.5± 13.6	365.3± 38.1	356.8± 80.4	424.8± 28.8	721.2± 30.3	722.7± 10.7	798.4± 6.7	169.8± 10.7	177.6± 1.4	180.0± 1.6
50 µM Cd	336.9± 8.2	279.5± 14.2	260.0± 11.5	257.2± 17.3	177.4± 5.8	168.2± 0.7	594.1± 11.0	456.9± 13.9	428.2± 8.9	166.0± 2.2	154.1± 2.4	145.3± 1.3
P. oryzae	256.5± 2.9	256.1± 10.6	200.8± 58.1	181.8± 1.9	145.8± 24.7	124.1± 21.6	438.39± 3.4	402.0± 11.2	325± 3.7	121.0± 7.03	112.4± 4.8	63.5± 1.8
50 µM Cd + *P. oryzae*	329.0± 5.3	355.3± 7.2	382.0± 9.9	360.2± 4.8	366.9± 20.4	429.81± 1.1	689.3± 1.5	722.2± 13.2	811.8± 8.7	166.2± 1.1	143.5± 2.4	173.6± 3.4

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





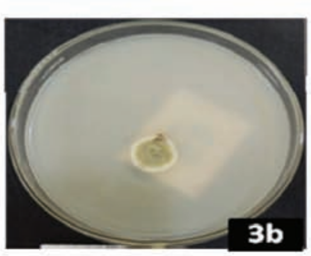


Fig 1. ***Pyricularia oryzae*** **(Broth)**
Fig 2. Plant growth : a- control, b- 50µM Cd, c- ***P.oryzae*****, d- 50µM+** ***P.oryzae***
Fig 3. Antifungal activity:3a- ***Pyricularia oryzae*** **(Control) ,**
3b- 50µM Cadmium+ ***Pyricularia oryzae***
Fig 4. Proline content of ***Oryza sativa*** **L. with different treatments**

4. Conclusion

Metal ions are toxic in nature but in low dose prevent fungal infection in plants. Cadmium application (50µM) to rice produced resistance that prevented *P. oryzae* infection. Chlorophyll and carotenoid, content increased in control and decreased in Cd and *P. oryzae* treated plants. In contrast, proline content increased with the day of treatment. In co-stress, *P. oryzae* and Cd at 7d, 15d and 21d intervals showed general improvement of plant growth accompanied with the increase of pigments as in control.

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