

Effect of Cadmium on Growth Parameters and Plant Accumulation in Different Rice (*Oryzasativa* L.) Varieties in Sri Lanka

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ABSTRACT: Rice (*Oryzasativa* L.) has the ability to uptake and accumulate cadmium (Cd) in different amounts within the plant parts. A pot experiment was conducted to investigate the variations of plant growth and levels of accumulation of Cd among eight rice genotypes (new improved varieties-NIVs and traditional varieties- TVs) grown under different soil Cd levels. Two soil Cd concentrations (50 and 100 mg/kg) were provided in the form of cadmium chloride and the effect of Cd on rice plant growth was measured with respect to the plant height, number of tillers, flag leaf chlorophyll content, leaf area of the flag leaf, and root dry weight. The soil without added Cd was considered as the control. The initial Cd concentration in the soil used was 1.958 mg/kg. The total amount of Cd in unpolished rice grains, shoots and roots were analyzed with Graphite Furnace Atomic Absorption Spectrophotometer and Flame Atomic Absorption Spectrophotometer. The results revealed that the rice variety, soil Cd level and interactions between rice variety and Cd level significantly affected ($p<0.05$) the plant height, flag leaf area, root dry weight and Cd accumulation. The variety Suwandel could not withstand soil Cd levels at 50 mg/kg and 100 mg/kg. Increasing soil Cd level reduced the plant height and root dry weight except in At 307. The variety Bg 300 was the lowest grain Cd accumulator without Cd chloride addition. Grain Cd accumulation in TV was high at 0 mg/kg level whereas NIV showed the highest accumulation at 50 and 100 mg/kg Cd levels. The shoot Cd level was increased with increasing soil Cd level. The Cd distribution within the rice plant is grains < shoots < roots.

Keywords: Accumulation, cadmium, growth, rice, varieties

INTRODUCTION

Heavy metals are important contaminants in the environment, especially in areas where there is high anthropogenic pressure. Cadmium is a non essential element and is the fifth most toxic metal to vertebrates and the fourth most toxic metal to vascular plants. It is believed that contamination of water sources and agricultural lands with fertilizers and agrochemicals for long periods of time is the main reason for accumulation of Cd. Bandara *et al.* (2008) and Premarathna *et al.* (2011) reported that the triple super phosphate (TSP) used by the Sri Lankan farmers carried 23.50 to 71.4 mg/Cd/Kg of P₂O₅. Also he further confirmed that

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commonly used weedicides in rice cultivation in Sri Lanka known as bispyribac sodium contains 0.5mg/l of Cd. Therefore, heavy Cd accumulation in agricultural soil can become dangerous to crops. When growing of plants in Cd polluted area, Cd is readily taken up by plant roots and translocated in to leaves in many plant species. Rice is the staple food in Sri Lanka and is considered to be a possible source of Cd intake (ref). The maximum permissible level of Cd in polished rice grain according to the Codex committee of the Food and Agriculture Organization of the United Nations (FAO) (ref) and by the WHO (Ref) is 0.4mg/kg. Wu & Zhang (2002) have reported that plant species and also genotypes within the species differ greatly in their tolerance to Cd stress. Sri Lanka has a genetically diverse rice collection with both traditional and new improved rice varieties and there is a lack of information available on grain Cd accumulation with different soil Cd levels and effect of Cd on growth performances of locally available rice genotypes. Thus this study was conducted to investigate the variations among rice varieties for Cd tolerance with respect to plant growth parameters and to determine the Cd uptake and accumulation by selected rice varieties grown in Sri Lanka.

MATERIALS AND METHODS

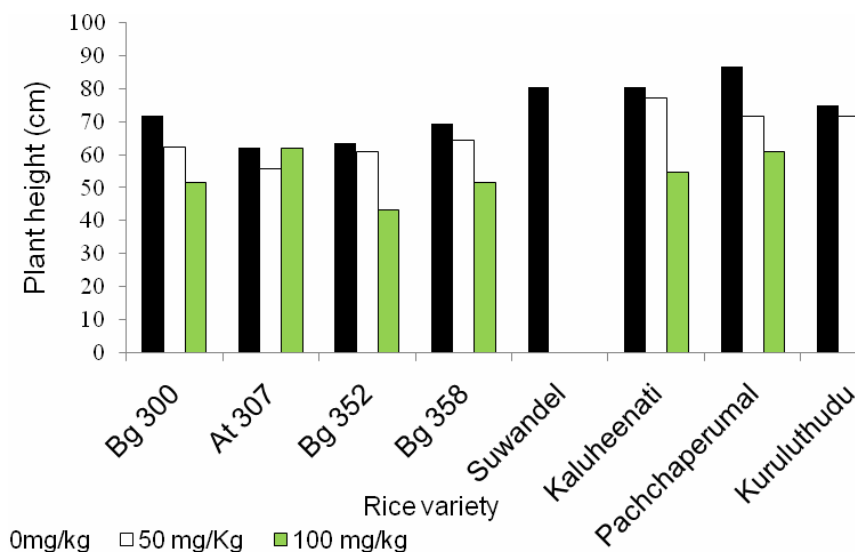
The study was performed as a pot trial in a plant house of the Department of Plant Sciences, Faculty of Agriculture, Rajarata University of Sri Lanka from April to August 2012 with eight rice (*Oryza sativa* L.) varieties. The soil was collected from the faculty field and samples were analyzed for soil basic properties and specially to detect the available Cd content. Twenty five kilograms of soil was placed in each pot and Cd in the form of $\text{CdCl}_2 \cdot 2 \frac{1}{2} \text{H}_2\text{O}$ was added to soil to obtain following Cd levels as; 50 mg Cd/kg of soil (T2) and 100 mg Cd/kg of soil (T3) and the soil receiving no external Cd was used as the untreated soil (T1). The pots once filled with soil were thoroughly mixed with water contaminated with exact amount of $\text{CdCl}_2 \cdot 2 \frac{1}{2} \text{H}_2\text{O}$ based on the treatment and submerged in water for 48 hours before the rice seedlings were transplanted in them. Eight different rice varieties were used in this experiment. Those were Bg300, At307, Bg352 and Bg358 as new improved rice varieties and *Suwadel*, *Kaluheenati*, *Pachchaperumal* and *Kuruluthudu* as traditional rice varieties. Seeds were surface sterilized with 20% clorox and soaked in water for 48 hrs and germinated in parachute trays. After 14 days, plants with two leaf stage were transplanted into the pots. The pot soil was maintained under submerged conditions during the whole growth period. The pots were arranged as a Two Factor Factorial Experiment with Completely Randomized Design with three replicates. Fertilizers were provided according to the Department of Agriculture, Sri Lanka recommendations. During the vegetative growth plant height, leaf area of the flag leaf, number of tillers, flag leaf chlorophyll content were measured. After the ripening stage, rice plants were uprooted and root dry weight was measured, Finally Cd accumulation in rice grain, shoot and root were measured using the following method. 0.3g of plant samples, were put on microwave digester (Model: Milestone start D) and digested by following modified US EPA method 3052. The sample Cd concentration was recorded using a GF-AAS (Model: GBC GF3000) at IFS, Kandy.

RESULTS

Effect of Cd on plant growth parameters

Experimental results of the plant height data (Fig. 1) showed that Bg 300, Bg 352, Bg 358, *Kaluheenati*, *Pachchaperumal* and *Kuruluthudu* reduced their plant height with increasing

soil Cd levels. Both Bg 300 and Bg 358 reduced their height significantly with the increasing soil Cd level. Even though Bg 352, *Kaluheenati* and *kuruluthudu* reduced their height with increasing soil Cd, the reduction of plant height from no addition of Cd to 50 mg/kg Cd was not significant. However, this reduction was significant from 50 mg/kg soil Cd to 100 mg/kg. Addition of Cd did not significantly affect the plant height of At 307. Even though 50 mg/kg Cd addition reduced the plant height of At 307, there was no significant difference in plant height for both no addition of cadmium salt and 100 mg/kg Cd addition.



In the Legend, show the black box to indicate 0 mg/kg (send me the excel file)

Fig. 1. Varietal response to Cd on plant height

Flag leaf chlorophyll content of different rice cultivars showed (Fig. 2) different responses with increasing soil Cd level. With the increasing soil Cd level, flag leaf chlorophyll content wasn't significantly reduced in Bg 300, At 307, Bg 358, *Kaluheenati* and *Pachchaperumal*. At 307, Bg 358, *Kaluheenati* and *Pachchaperumal* showed higher SPAD value with 50 mg/kg soil Cd than in both no addition of Cd and 100 mg/kg Cd treatments. But Bg 352 and *Kuruluthudu* showed the reduction of flag leaf chlorophyll content with increasing soil Cd levels. Both of these two rice varieties showed the highest chlorophyll content at zero addition of cadmium chloride and the lowest chlorophyll content at 100 mg/kg Cd treatment.

The leaf area results revealed that rice variety, soil Cd level and variety x Cd interaction significantly affected average leaf area of the eight tested rice varieties. Increasing soil Cd level reduced the average leaf area of all tested rice varieties except in *Suwandel*.

This reduction was highly significant for Bg 300 and Bg 358. When the soil Cd level changed from no addition of cadmium chloride to 50 mg/kg soil Cd, the reduction of leaf area was significant for both At 307 and *Pachchaperumal*. However, this reduction was not significant for Bg 352, *Kaluheenati* and *Kuruluthudu*. Although the reduction of leaf area was not

significant when soil Cd is increased from 50 to 100 mg/kg for At 307, this reduction was significant for Bg 352 and *Kuruluthudu*.

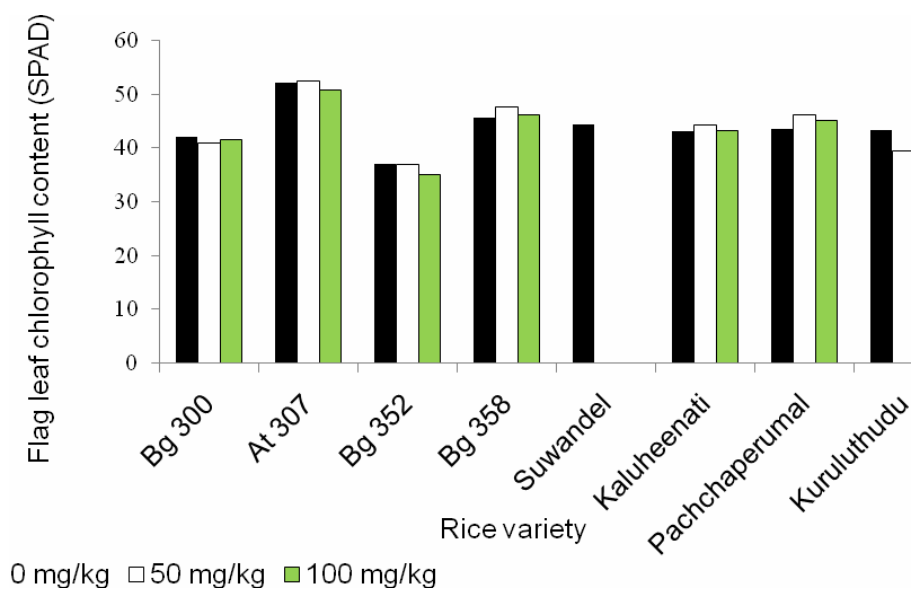


Fig. 2. Varietal response of Cd on flag leaf chlorophyll content

Fig. 3 shows the variation of leaf area with tested three soil Cd levels.

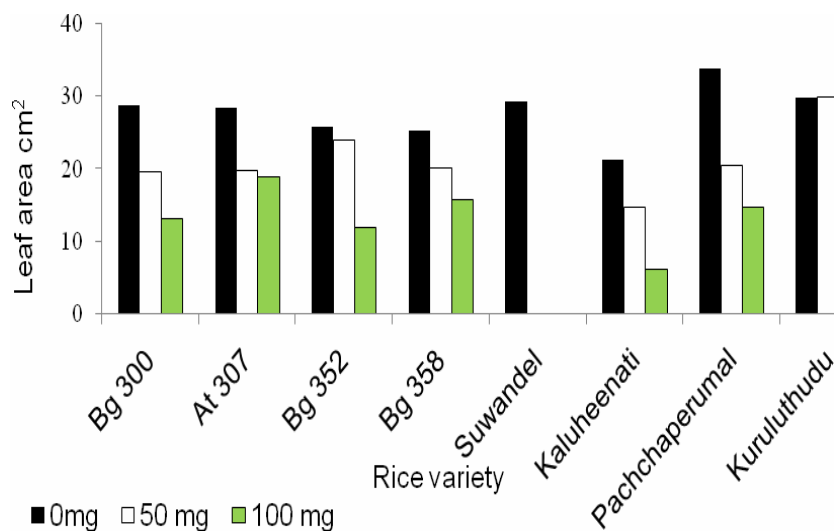


Fig. 3. Variation of leaf area with different soil Cd levels

When considering the tiller number, increasing soil Cd level reduced the number of tillers. This reduction was not significant for Bg 300, At 307, *Kaluheenati*, *Pachchaperumal* and *Kuruluthudu* (Fig. 4). For Bg 352 and Bg 358 there was a significant reduction of tiller number when the soil Cd level is increasing. When the soil Cd level is changing from 0 mg/kg to 50 mg/kg both Bg 352 and Bg 358 reduced the number of tillers, but this reduction was not significant for both these two varieties. However, there was a significant reduction of tiller number observed in these two rice varieties when the soil Cd changed from 50 to 100 mg/kg.

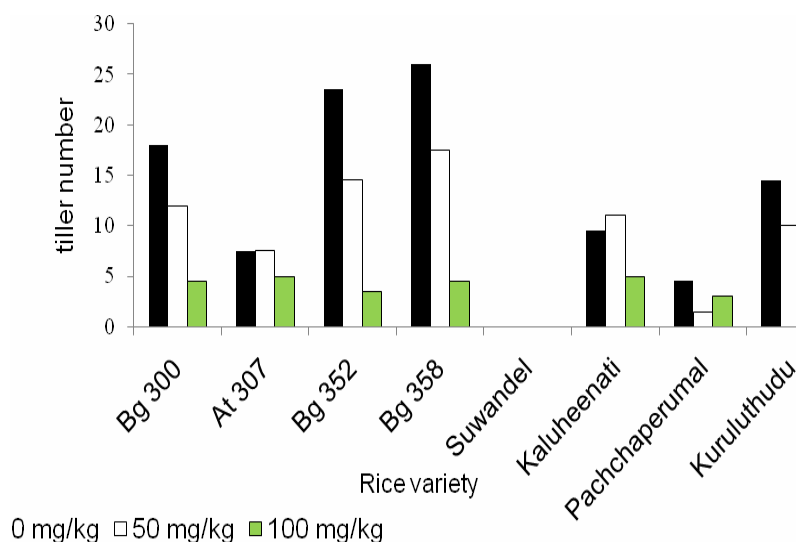


Fig. 4. Variation of number of tillers with different soil Cd levels

Root dry weight results (Fig. 5) revealed that, rice plants reduced their root dry weight when the soil Cd level is increasing. Bg 358 significantly reduced their root dry weight with increasing soil Cd level. Similarly Bg 300 reduced their root dry weight when soil Cd was changed from 0 to 50 mg/kg. However, this reduction was not significant but the increasing soil Cd from 50 to 100 mg/kg caused the significant reduction of root dry weight for Bg 300. Variety At 307 responded differently to the soil Cd level than other varieties. This variety has significantly reduced their root dry weight when the soil Cd level changed from 0 to 50 mg/kg. Although this variety increased the root dry weight from 50 mg/kg Cd treatment to 100 mg/kg treatment numerically, this increase was also not significant. Though Bg 352 significantly reduced the root dry weight when the soil Cd level is 50 mg/kg, *Kaluheenati*, *Pachchaperumal* and *Kuruluthudu* also behaved in similar pattern. Further, these four rice varieties showed the reduction of root dry weight when the soil Cd level was 50 to 100 mg/kg but this reduction was also not statistically different. Fig. 6 shows the variation of root dry weight with increasing soil Cd level.

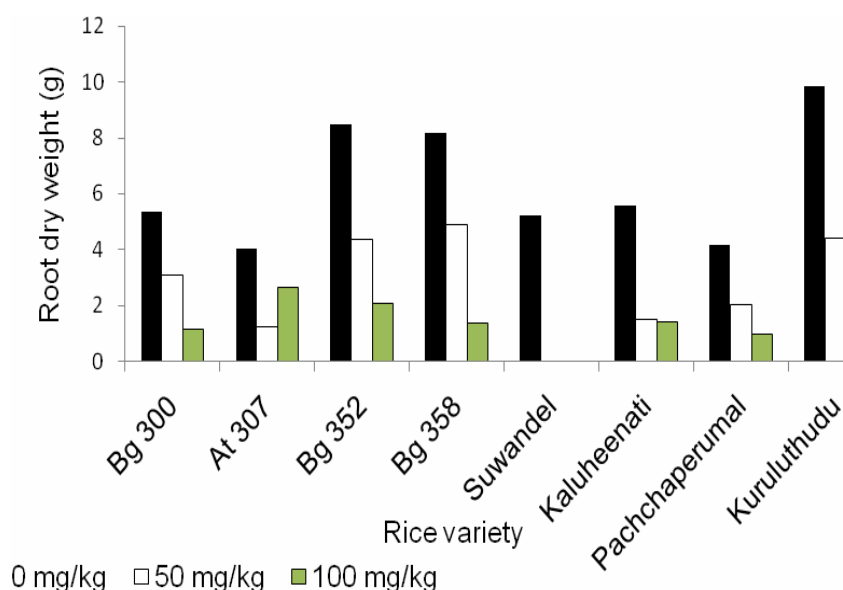


Fig. 5. Varietal response to Cd on root dry weight

Varietal Response to Cd Accumulation and Distribution within the Body of Rice Plant

The experimental results of the average Cd accumulation of unpolished rice grains of the eight rice cultivars with three different soil Cd levels are shown in Fig. 7. According to the results, rice variety, soil Cd levels and the interaction between rice variety and soil Cd levels significantly affected grain Cd accumulation. The total grain Cd content in each rice variety of Bg 300, At 307, Bg 352, Bg 358, *Kaluheenati*, *Pachchaperumal* and *Kuruluthudu* were statistically different with the three soil Cd levels. Generally, the mean grain Cd levels increased with increasing soil Cd levels except variety in Bg 300. In Bg 300, the grain Cd content in 100 mg/kg soil Cd was lower (2.26 mg/kg) than the 50 mg/kg (2.719 mg/kg) soil Cd. In At 307, Bg 352, Bg 358, *Kaluheenati*, *Pachchaperumal* and *Kuruluthudu* grain Cd content increased with increasing soil Cd concentration. The mean Cd content in all tested rice shoots except in variety *Suwandel* was highly statistically different at all three soil Cd treatments. For all tested rice varieties, the mean shoot Cd content was increased significantly with increasing soil Cd levels.



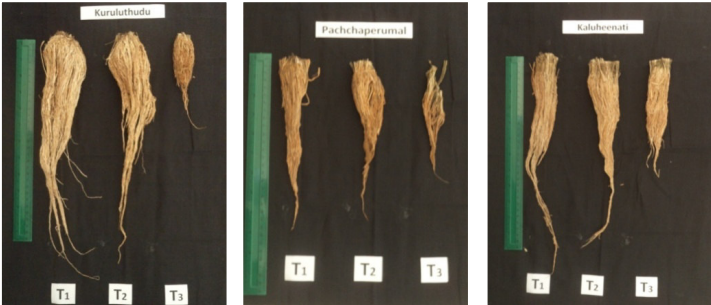


Fig. 6. Varietal response to Cd on root dry weight

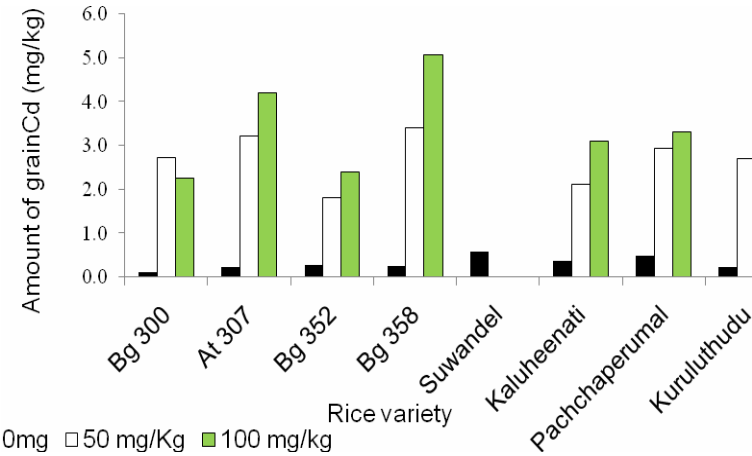


Fig. 7. Average grain Cd content in rice varieties with different soil Cd levels

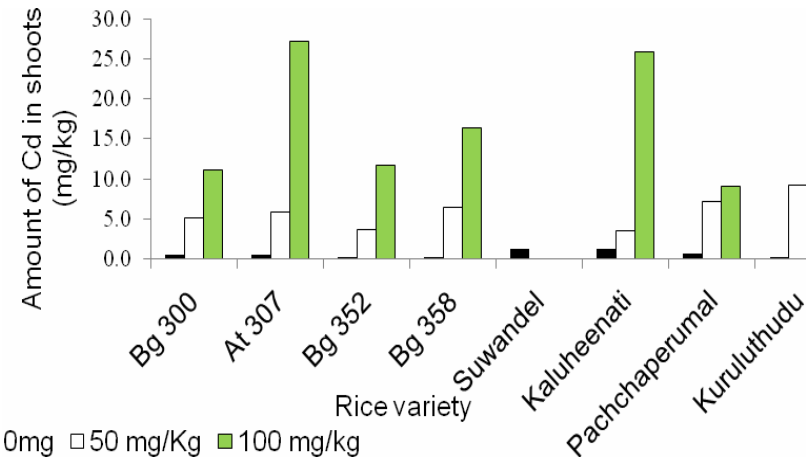


Fig. 8. Average shoot Cd content in rice varieties with different soil Cd levels(you have not mentioned about this figure in the text)

Cd content in roots of all tested rice varieties except in *Suwandel* was highly statistically different at the tested soil Cd levels. Results obtained from the study showed that variety Bg 300, At 307 and *Kuruluthudu* have increased their Cd accumulation with increasing soil Cd levels. Other rice varieties except *Suwandel* accumulated lower amount of Cd in their roots at 100 mg/kg soil Cd treatment than the 50 mg/kg soil Cd treatment.

It has been reported that the average Cd accumulation in rice roots were much higher than in stems and leaves and rice grains (Liu *et al.*, 2003). Our study too confirmed this phenomenon; it showed that all the tested rice varieties accumulated Cd in the sequence of grain < shoot < root for every soil Cd level treated i.e. rice grains accumulated the lowest and the roots accumulated the highest. Although *Suwandel* variety completely died with 50 and 100 mg/kg soil Cd levels, it showed the same sequence with zero addition of cadmium chloride. So the results obtained from the study indicated that a great proportion of Cd taken up by rice plant was retained in roots. The Cd concentration fell rapidly from root to grains along rice plants and only a very small proportion of Cd taken by root was transferred to grain. So the concentration of Cd in grain was very low compared with other parts of the rice plant.

DISCUSSION

It has been shown that Cd is strongly phytotoxic and causes growth inhibition and even plant death (Sanita di Toppi&Gabrielli, 1999). In general, Cd in plants reduces the growth both in roots and stems. This effect is partly due to the suppression of the elongation growth rate of cells, especially in the stem, because of an irreversible inhibition exerted by Cd on the proton pump responsible for the process (Aidid&Okamoto, 1992, 1993; cited by Sanita di Toppi&Gabrielli, 1999). Liu *et al.*, 2007 did the same experiment using six rice cultivars with 100 mg/kg soil Cd and concluded that toxicity effect of Cd on plant height is varietal dependent; the present study also proved this result. Further, Shao Guo-sheng *et al* (2007) did a hydroponic study to investigate Cd accumulation and its toxicity in rice on the *Brittle Culm1 (bc1)* gene-a fragile rice mutant and its wild type with 0, 0.1, 1.0 and 5.0 $\mu\text{mol/L}$ Cd levels and they concluded that both rice genotypes substantially inhibited the plant growth under high Cd levels (at 1.0 and 5.0 $\mu\text{mol/L}$ Cd levels).

Liu *et al.* (2007) have reported that the effect of Cd on dry matter accumulation of rice plants differed with rice cultivar, rice growth stage and plant organ. Results of the present study also revealed significant root dry weight reduction between no addition of cadmium chloride and 50 mg/kg Cd treatment except in Bg 300. The same result was further observed with no addition of cadmium chloride and 100 mg/kg Cd treatment except in At 307. Further there was a significant difference among rice genotypes with no addition of cadmium chloride and 50 mg/kg Cd treatments, but there was no observed significant difference among plant genotypes at 100 mg/kg soil Cd treatment. Roots responded differently to the soil Cd stress, here some plant genotypes showed comparatively high tolerance (Bg 300, At 307 and *Kuruluthudu*) than others to 100 mg/kg soil Cd treatment while some genotypes showed the slight toxicity (Bg 352, Bg 358, *Kaluheenati* and *Pachchaperumal*). Yu-jing *et al.* (2004) also observed significant difference of rice root biomass with 0 mg/kg, 5 mg/kg and 10 mg/kg soil Cd addition. They further reported that the addition of 10 mg/kg Cd to the soil significantly reduced the root bio mass.

When the growing medium Cd concentrations are different, rice plant can accumulate different amounts of Cd in their grains. Different literature have reported different levels of rice grain Cd with different Cd treatments. As an example, Jinet *al.* (2009) reported that the

mean grain Cd in unpolished rice grains of 110 cultivars as 0.022 mg/kg for paddy soils which contains 0.15 mg/kg soil Cd. Areo&Ae, 2003 have reported that the mean grain Cd content in 31 different rice cultivars as 2.14 mg/kg (for brown rice) with 0.9 mg/kg soil Cd and 3.11 mg/kg with 7.4 mg/kg soil Cd. Further they have reported that the variety Rathal (a traditional variety grown in Sri Lanka) accumulates 2.12 and 3.34 mg/kg Cd in their grains in the same study. When comparing these values with our results, the mean grain Cd content in 100 mg/kg soil Cd (3.376 mg/kg) is nearly similar to the grain Cd content in Rathal (3.34 mg/kg) for 7.4 mg/kg soil Cd. In some parts of China, soil Cd content is up to 26 mg/kg and the highest Cd in rice grains was reported as 2.4 mg/kg (Jin, 2002: cited by Yu-jing *et al.*, 2004).

The maximum permissible level of Cd in polished rice grain according to the “Codex Alimentarius” Committee of the Food and Agriculture Organization of the United Nations (FAO) and by the World Health Organization (WHO, 2009) is 0.4 mg/kg. However, Bg 300, At 307, Bg 352, Bg 358, *Kaluheenati* and *Kuruluthudu* did not exceed the WHO permissible level, but *Suwandel* and *Pachchaperumal* exceeded the permissible level with no addition of cadmium chloride.

During the last decade, a significant increase in the number of CKD patients from the North Central Province (NCP) region of Sri Lanka has become a major environmental health issue of national concern. Exposure to a combination of factors that are toxic to the kidney seems to cause this kidney disease. Toxic factors identified up to now include arsenic, cadmium and nephrotoxic agrochemicals (WHO Mission report, 6-8th June 2011). In some areas in China and Thailand, production of highly Cd polluted rice and renal dysfunctions among populations were reported (Nordberg *et al.*, 1997; Jin *et al.*, 2002; Honda *et al.*, 2010).

CONCLUSIONS

In the present experiment, the rice plants exposed to higher levels of Cd showed growth inhibition in plant height, chlorophyll content in flag leaf, leaf area, number of tillers and root dry weight.

Cd concentration in different cultivars responded differently to increasing soil Cd levels. The Bg 300 accumulated lower amount of Cd in normal soil conditions, therefore, due to this phenomenon it could be concluded that Bg 300 is most safe for human consumption than the other tested rice varieties. Further Bg 300 accumulated low amount of Cd in their grains at the 100 mg/kg soil Cd. Therefore, this variety is most suitable to grow in highly Cd polluted locations among other tested varieties of rice.

Roots accumulate the highest amount of Cd than shoots and grains and distributed Cd as grains < shoots < roots for all tested soil Cd levels. Both new improved and traditional rice varieties behaved similarly.

Among the tested varieties Bg 300, At 307 and *Kuruluthudu* were the most tolerant rice varieties while Bg 352, Bg 358, *Kaluheenati* and *Pachchaperumal* were the less tolerant varieties. Among tested rice varieties, At 307 was the highly tolerant variety to soil Cd stress because At 307 could compensate both shoot and root growth when soil Cd level was 100 mg/kg. Rice variety *Suwandel* is a highly sensitive variety than the other tested rice varieties for both 50 and 100 mg/kg soil Cd levels because these two soil Cd levels are toxic to this variety. Thus this variety could not withstand at both 50 and 100 mg/kg soil Cd levels.

Without addition of external Cd as cadmium chloride, traditional rice varieties always accumulated the highest amount of Cd within all plant parts (i.e rice grains, shoots and roots) than new improved rice varieties. Therefore, the traditional rice varieties can accumulate a considerable amount of Cd within their body than new improved rice varieties with normal soil conditions.

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