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ORIGINAL ARTICLE

Cadmium Tolerance of Rice (*Oryza Sativa* L.): Effects on Plant Growth and Physiology

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Abstract

The effects of cadmium (Cd) toxicity on plant growth and physiology were investigated in rice (*Oryza sativa* L.) grown in sand medium. *Suwandel* and Bg300 rice varieties were evaluated against 0, 0.5 and 3 mg kg^{-1} Cd levels using thirteen plant growth and physiological parameters. The only parameter that influenced by the rice variety and Cd level interaction effect was the transpiration rate. All other parameters were not changed significantly by the said interaction effect. Cadmium level of 3 mg kg^{-1} significantly decreased the shoot dry weight and photosynthetic rate. It also increased the transpiration rate of *Suwandel* variety. Hence, 3 mg kg^{-1} level was the most critical one for shoot dry weight, photosynthetic rate and transpiration rate. However, Cd levels were not significantly changed plant height, leaf area, root dry weight, death leaf percentage, relative leaf chlorophyll content, stomatal conductance to H_2O, intercellular CO_2 concentration, computed leaf temperature, cell membrane permeability and relative water content. This concluded that Bg300 and *Suwandel* can compensate the toxic effects exerted by 0.5 and 3 mg kg^{-1} Cd levels without affecting the plant growth and physiology.

Keywords: *Plant Cd responses, Growth, Photosynthesis, Water relations*

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1. Introduction

Cadmium is a widely known heavy metal which can be extremly toxic to plants, animals and humans due to its high mobility (Su et al. 2005). Contamination of agricultural soils by Cd has become a serious problem in some parts of the world due to its bioaccumulation (Herawati et al. 2000). According to the literature, application of phosphate fertilizers, manures, sewage sludge, agrochemicals and polluted irrigation water to crop fields can lead to to increased Cd content in agricultural soils (Williams and David 1973; Cox 1986; He and Singh 1994; Smith 1994; Siamwalla 1996; Wong et al. 2002).

Different plant species as well as their genotypes show differential responses to Cd stress (Wang 2002; Wu and Zang 2002). When absorbed by plants, Cd can result in a variety of toxic effects due to imbalance of metabolic activities. In general, the well-known damage symptoms that occur in plants induced by Cd are leaf chlorosis, leaf roll (di Toppi and Gabrielli 1999), necrosis and red-brown coloration of leaf margins or veins (Prasad 1995; cited by Wójcik and Tukiendorf 2005). It has been reported that, Cd alters the cell membrane permeability (Kabata-Pendias and Pendias 2001; Wahid et al. 2007), tissue water balance (Singh and Tewari 2003) and damages photosynthetic apparatus especially photosystem I and II (Siedlecka and Krupa 1996) and light harvesting complex II (Krupa 1988). Further, Cd is reported as a cause to decrease carbon assimilation, generate oxidative stress (Yadav 2010), inhibits chlorophyll synthesis (Shukla et al. 2008), change nitrogen metabolism (Kastori et al. 1997), damage root tips, reduce nutrient uptake, impair photosynthesis and finally, inhibit plant growth (Wójcik and Tukiendorf 2005).

Heavy metal pollution is one of the recent threats in Sri Lanka. Since Cd is a well-known nephrotoxic element, some researchers claim Cd as a probable cause for Chronic Kidney Disease of unknown etiology (CKDue) in Sri Lanka. Rice is considered as one of the crops with high Cd uptake and accumulation (Chaney et al. 2004). Since rice is the staple food for Sri Lankans, there is a great concern in the country on the amount of Cd accumulated in rice. Sri Lankan rice varieties show a tolerance to many useful rice stresses including ion toxicity, salinity, drought and some other adverse soil conditions (Arachchi and Wijerathna 2008). However, the differences among local rice varieties for Cd tolerance are still unclear. Therefore, a proper understanding of growth and physiological mechanisms of local rice germplasm under Cd stress is essential towards developing Cd tolerant rive varieties.

The European Community (1986) has recommended 1 and 3 mg kg^{-1} as the lower and upper limits of Cd for agricultural fields respectively. Literature indicates elevated levels of soil Cd in some crop fields and in lake sediments of the country compared to the European Community set standards. Such sites are in Haputale (3.86 mg kg^{-1}), Sedawatta (3.28 mg kg^{-1}) (Premarathna et al. 2011) and in dry zone of the country (5 mg kg^{-1}) (Chandrajith et al. 2012). Moreover, in the dry zone of Sri Lanka, farmers generally incorporate lake sediments to their crop fields to get a better crop growth, hence, there is a chance of elevating Cd levels in the dry zone crop fields.

Therefore, identification of Cd tolerance characters of local rice varieties within the recommended soil Cd levels by the European Commission will give a chance to utilize local gene pool for the development of Cd stress tolerance in rice. Not only that, it has the potential to direct introduction of those into the farmer fields where the Cd problem is occurred. Therefore, this study was designed to evaluate growth and physiological responses of Bg300 and *Suwandel* rice varieties at the recommended upper limit of Cd set by the European Community in 1986, with the objectives of finding the capability of growing such rice varieties into Cd prevalent areas of the country and to further utilize them in local rice breeding programs to develop Cd tolerance rice varieties.

2. Materials and Methods

Pot Preparation:

The study was performed as a pot trial in the plant house of the Faculty of Agriculture, Rajarata University of Sri Lanka. Clean, washed and air-dried river sand was used for the experiment. Ten kilograms of air-dried sand was placed in each pot. Cadmium in the form of $CdCl_2.2$ (½) H_2O was added to the prepared pots to obtain two Cd levels as 0.5 and 3 mg kg^{-1}. A control was established without external application of Cd.

Urea, Triple super phosphate and Murate of potash were added into each pot as recommended by the Department of Agriculture, Sri Lanka (Fertilizer usage in paddy cultivation 2010) and Yoshida nutrient media (Yoshida et al. 1976) was used to provide micronutrients. The contents in pots were thoroughly mixed to make homogenized condition and submerged in water (nearly 5 cm above the sand surface) for two days before the rice seedlings were transplanted into the pots.

Planting Materials and Experimental Design:

Based on the previous findings of Herath et al. 2014, seeds of two rice varieties were used in the present experiment. One is Bg300; an improved rice variety with lowest grain Cd accumulation ability when grown in normal paddy field soil in Anuradhapura. The second variety is *Suwandel*; a traditional rice variety having the highest grain Cd accumulation ability when grown in normal paddy field soil in Anuradhapura.

The treatments were assigned as two factor factorial experiment. Rice varieties were considered as the first factor and the Cd levels were taken as the second factor. All factor combinations were replicated three times resulting 18 pots. The subjective pots were arranged in the plant house as Complete Randomized Design.

Seeds of the said rice varieties were washed, surface sterilized with 10 % Clorox and soaked for 24 hours at room temperature and germinated on sand containing Petridishes. After 10 days, four uniform healthy seedlings were transplanted into the prepared pots. Pots were irrigated and approximately 5cm of water above the sand surface was maintained during the whole growth period.

Measuring plant parameters:

Following parameters were monitored in all plants at the age of one month. Plant height (height from the base of the plant to the tip of the first fully expanded leaf of the plant) was measured using meter ruler. Leaf area of first fully expanded leaf of each plant was measured using a leaf area meter (Model: CI – 202). Root and shoot dry weights were measured after uprooting and oven drying (at 60 ^{0}C) until a constant weight was obtained (Januškaitienė 2010). Death leaf percentage of all plants were calculated manually by counting total number of leaves and number of dead leaves in each plants. Average relative leaf chlorophyll content of first fully expanded leaf of all plants was measured using a Soil Plant Analysis Development (SPAD) chlorophyll meter (Model: SPAD – 502 plus).

The following photosynthesis related parameters were measured using portable photosynthesis system LI-6400 XT (LI-COR, USA). Photosynthetic rate (Ph) (µmol CO_2 m^{-2} s^{-1}), stomatal conductance to H_2O (mol H_2O m^{-2} s^{-1}), intercellular CO_2 concentration (Ci) (µmol CO_2 mol^{-1}), transpiration rate (Tr) (mmol H_2O m^{-2} s^{-1}) and computed leaf temperature (CT) (^{0}C) of first fully expanded leaf were measured after stabilizing the machine for ambient CO_2. Measurement conditions were; air flow rate- 400 µmol s^{-1}, expected leaf temperature- 30 ^{0}C, leaf area- 1 cm^2 (i.e. the leaf area covered by the leaf chamber when it was inserted) and maximum photosynthetic active radiation (PAR)-1500 µmol m^{-2} s^{-1}. The LI 6400- 02B LED source was used to provide desired PAR intensity.

Cell membrane permeability was evaluated as a function of cellular electrolyte leakage (EL) as practiced by Park et al. (2010) with few modifications. The first fully expanded leaf of the plant was detached and weighted (M). Then, the leaf was cut in to approximately 1cm length pieces. The leaf pieces were washed in deionized water and blot dried on tissue papers. Such pieces were placed in 20 mL of deionized water containing polypropylene tubes at room temperature for 1 hour with gentle shaking. Then, electrical conductivity (EC) of bathing solution (EC1) was measured using an EC meter. The total EL was measured after freezing subsequent samples at -40 ^{0}C for 8 hours (EC2). Then, EL was expressed using following equation; [(EC1/EC2 X M) X 100].

Relative water content (RWC) of stressed tissues was measured as explained by Amirjani (2010) with few modifications. Briefly, the first fully expanded leaf was detached from the plant at pre-dawn period of the day and the RWC was determined as a percentage, using following equation: RWC = [(Wi – Wd)/(Wf – Wd)] X 100; where, Wi is the initial leaf weight, Wd is the leaf dry weight and Wf is the full turgid leaf weight.

Statistical analyses:

Data analysis was performed with the Statistical Analysis System (SAS) software (Version 9.0). Before performing the analysis, all variables were checked for the normality. Then, all recorded plant growth and physiology data were analyzed using General Liner Model procedure. Adjusted values of Bonferroni means were used to compare mean effects. Significant level of 0.05 was used in interpreting the results.

3. Results and Discussion

Effect of Cd on Rice Plant Growth:

The statistical analysis showed that there was no significant interaction effect of rice variety and Cd level on plant height, leaf area, shoot dry weight, root dry weight and death leaf percentage.

Growth responses of Bg300 and *Suwandel* for different levels of Cd in growing medium are shown in Fig. 1. Results revealed that there is a significant impact of rice variety on the plant height (Fig. 1a). Results of the present study confirms that rice variety *Suwandel* has the highest plant height compared to Bg300.

Leaf area of rice varieties were not affected significantly from both Cd and variety differences (Fig. 1b). However, there was a significant impact of Cd on shoot dry weight (Fig. 1c). At both 0.5 and 3 mg kg^{-1} Cd treatments, shoot dry weight was reduced significantly compared to the control. Further, there was no significant difference observed in shoot dry weight between Bg300 and *Suwandel* varieties for Cd treatments.

Root dry weights were not significantly affected either by Cd treatments or rice varieties. The respective mean values are presented in Fig 1d. Further, the death leaf percentage was not affected significantly by Cd treatments and rice varieties (Mean values are in Fig. 1e).

Among all the tested plant growth parameters, Cd stress was significantly affected only for shoot dry weight. Here, a significant growth inhibitory effect was observed when compared to the control. However, a slight growth acceleration was observed in Bg300 for both shoot and root dry weights when the growing medium Cd concentration was increased from 0.5 to 3 mg kg^{-1}. According to the mean values presented in Fig.1, two distinct growth patterns were observed in Bg300 and *Suwandel*. Rice variety Bg300 has shown growth retardation in 0.5 mg kg^{-1} Cd level and the growth was accelerated with 3 mg kg^{-1} Cd except in the leaf area. In contrast, *Suwandel* showed growth inhibition in both 0.5 and 3 mg kg^{-1} Cd treatments except shoot dry weight and death leaf percentage. However, the degree of this inhibition was higher in 3 mg kg^{-1} Cd than in 0.5 mg kg^{-1}. This postulates the affinity of Bg300 to compensate the toxicity of 3 mg kg^{-1} Cd than the *Suwandel*. Thus, *Suwandel* was less tolerant to 0.5 and 3 mg kg^{-1} Cd than Bg300 according to the plant growth parameters. However, this should be further investigated with more plant growth parameters.

It was reported that plants exposed to Cd showed a series of growth and physiological disorders (Jing et al. 2005; Liu et al. 2007; Guo et al. 2016). This study also shows a growth inhibition in the form of shoot dry weight reduction irrespective to the rice variety. This may be due to the entrance of heavy metals into the plant cell with the increase of heavy metal concentration in the growing medium. As a result, cellular metabolism can be inhibited due to heavy metal toxicity (Xie et al. 2014).

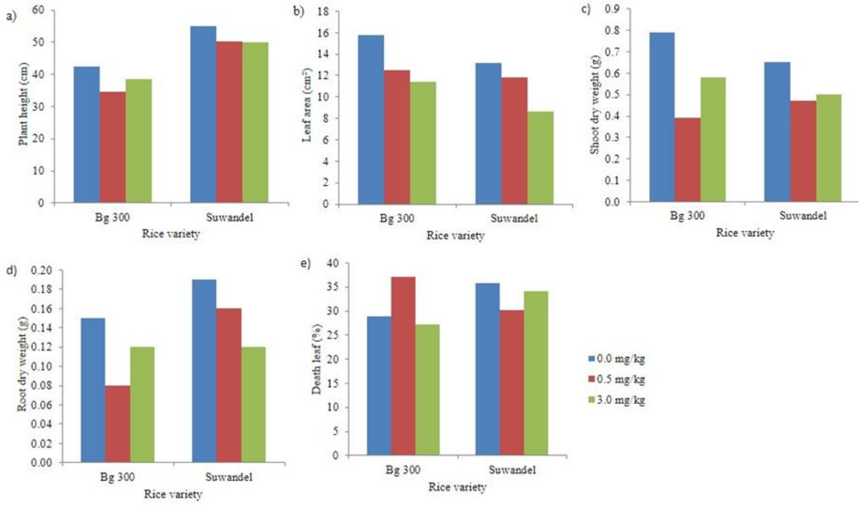
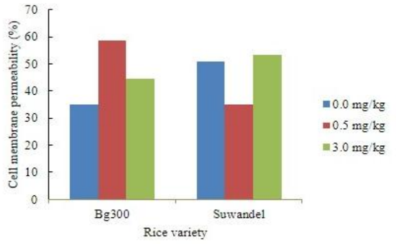


Figure 1: Effect of Cd (mg kg^{-1}) on growth of rice plant: a) Plant height, b) Leaf area, c) Shoot dry weight, d) Root dry weight, e) Death leaf percentage



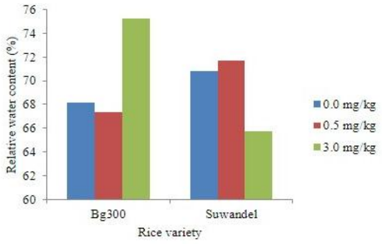


Figure 3: Effect of Cd on cell membrane permeability

Figure 4: Effect of Cd on RWC

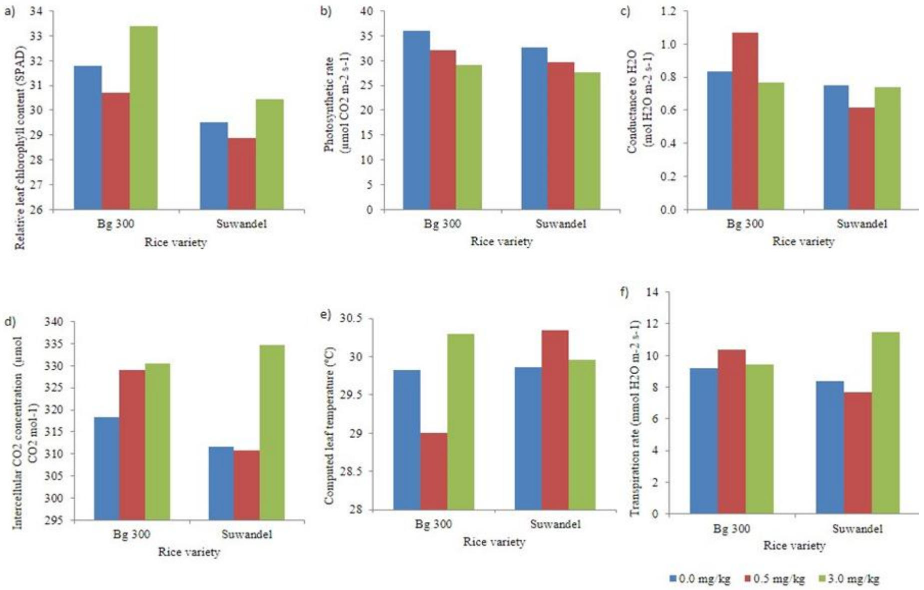


Figure 2: Effect of Cd on chlorophyll content and photosynthetic related parameters: a) Relative leaf chlorophyll content (SPAD), b) Photosynthetic rate (Ph), c) Stomatal conductance to H_2O, d) Intercellular CO_2 concentration (Ci), e) Computed leaf temperature (CT), f) Transpiration rate (Tr)

Poschenrieder et al. (1989) and Wan and Zhang (2012) observed that Cd in cells can binds with cell walls and middle lamella. It enhances affinity of making cross-linkages between Cd and cell wall components and inhibits the cell growth. Moreover, Cd causes metabolic dysfunctions in plant cells by enhancing the production of reactive oxygen species (ROS) (Pérez-Chaca et al. 2014), inhibition of photosynthesis (Jing et al. 2005; Paunov et al. 2018) and reduction of nutrient uptake (Johnson et al. 2011; Jibril et al. 2017). These altered processes potentially causing the observed growth retardation of rice plants due to Cd stress.

As stated above, when Bg300 was exposed to 3 mg kg^{-1} of Cd, it induced plant growth in terms of plant height, shoot and root dry weights and in death leaf percentages (Fig. 1c, 1d and 1e, respectively). This is consistent with the previous findings of positive impact of Cd on rice root growth as explained by Li et al. (2014) and plant height improvement of tomato seedlings (Jing et al. 2005). This growth acceleration effect is possible due to production of glutathione, oxalic acid, citrate and metal binding proteins to compensate heavy metal toxicity of plants when plants get exposed to Cd as observed by Zhang et al. (1999).

It is a known fact that root growth is more sensitive to heavy metals than shoot growth. However, present study has shown a significant shoot growth retardation by Cd while the root growth was not affected. In support to the above finding, Obroucheva et al. (1998) also stated that there was a significant heavy metal accumulation in roots compared with shoots. The same phenomenon was reported for Sri Lankan rice varieties at high Cd concentrations (i. e. 50 and 100 mg kg^{-1}) compared to the present study (Herath et al. 2014). Therefore, rice roots can contain a considerable amount of Cd than in shoots. This low shoot Cd may be the reason to induce shoot growth as observed by the study. Therefore, it can be suggested that the root growth may not necessarily be more sensitive to Cd than shoot growth of rice due to the differential partitioning of Cd.

Effect of Cd on Photosynthesis and Related Parameters:

Fig. 2 shows the changes of photosynthesis related parameters to tested Cd levels. Findings revealed that there is no significant interaction effect of rice variety and Cd on relative leaf chlorophyll content (Fig. 2a). Also, the relative leaf chlorophyll content was not significantly affected either by the Cd levels or rice varieties.

The interaction effect of rice variety and Cd level was not significant for Ph. But, Cd showed a significant impact on Ph irrespective of the variety. Rice varieties grown under 3 mg kg^{-1} Cd showed a significantly low Ph (28.48 µmol CO_2 m^{-2} s^{-1}) compared to the control (Fig. 2b). There was a reduction in Ph in 0.5 mg kg^{-1} Cd treatment (30.96 µmol CO_2 m^{-2} s^{-1}) compared to its control (34.38 µmol CO_2 m^{-2} s^{-1}). However, this reduction was not significant.

Significant interaction effect was not observed between rice variety vs. Cd level on stomatal conductance to H_2O. But, there was a significant effect of rice variety to the stomatal conductance. Here, Bg300 showed a significantly higher stomatal conductance to

H_2O value (0.91 mol H_2O m^{-2} s^{-1}) than the *Suwandel* (0.69 mol H_2O m^{-2} s^{-1}) (Fig. 2c). Furthermore, no significant differences were found in variety Cd interaction effect and main factor effects for Ci and CT of rice (Fig. 2d and 2e, respectively).

A significant variety and Cd interaction effect was obtained for Tr. Here, *Suwandel* showed significant changes of Tr in different Cd treatments. It showed a significantly high Tr (11.49 mmol H_2O m^{-2} s^{-1}) at 3 mg kg^{-1} Cd treatment compared to the control (8.40 mmol H_2O m^{-2} s^{-1}). Further, the Tr values of *Suwandel* was increased significantly when Cd concentration changed from 0.5 mg kg^{-1} (7.69 mmol H_2O m^{-2} s^{-1}) to 3 mg kg^{-1} (11.49 mmol H_2O m^{-2} s^{-1}). In contrast, Bg300 did not show any significant changes of Tr to tested Cd levels. This highlighted the higher sensitivity of *Suwandel* for the Tr than in Bg300 (Fig. 2f).

Cadmium toxicity caused a notable reduction in Ph in different plant species (Sawhney et al. 1990). According to the present study, the rice varieties exposed to 3 mg kg^{-1} Cd concentration confirmed this finding. The observed decrease of Ph in Cd treated rice plants may not be due to the relative leaf chlorophyll content as expressed by SPAD values in the study. However, Jing et al. (2005) found a link between Ph values and SPAD values as the reduction of Ph is due to the low SPAD values. Therefore, it may be assumed that the decrease in Ph is not only due to the SPAD values, but also due to the inhibition of steps in the photosynthesis cycle as explained by Prasad and Strzalka (1999). Moreover, slight increase in intercellular CO_2 concentration was observed during the present study when Cd levels were increased. A similar result was obtained by Jing et al. (2005) for tomato seedlings. However, further evidences are needed to have a better understanding of physiological mechanisms of the Cd toxicity on the photosynthesis.

Further, it has been reported that leaf temperature can be used effectively as a bio-indicator to monitor metal polluted soils, because heavy metal stress can increase leaf temperature by 1 - 3 ^{0}C (Horler et al. 1980; Suresh et al. 1989; Thakur et al. 2012). In the present study, a slight increase of leaf temperature was observed with 3 mg kg^{-1} Cd treatment compared with its control in both rice varieties. But, the temperature difference was lower than 1 ^{0}C. Thus, it can be postulated as these two rice varieties cannot be used effectively to identify Cd polluted sites when Cd content is similar or lower than the 3 mg kg^{-1} in terms of leaf temperature.

Effect of Cd on Cell Membrane Permeability and RWC:

Cell membrane permeability and RWC were not significantly affected by variety Cd interaction (Fig. 3 and Fig. 4, respectively). The tested Cd levels did not make significant changes in cell membrane permeability of Bg300 and *Suwandel*. However, there was a significant varietal effect on the cell membrane permeability. *Suwandel* showed a significantly high cell membrane permeability compared to Bg300. This further revealed a significant cell membrane stability in Bg300 compared with *Suwandel.* Moreover, no significant main factor effects were observed in RWC of leaf tissues.

Thus, RWC was not significantly affected either by the tested Cd levels or by the rice varieties.

Heavy metals are one of the known factors to disturb plant water relations by interfering with transpiration rate and stomatal conductance (Barcelo and Poschendrier 1990; Kastori et al. 1992; Costa and Morel 1994). Calcium mediated signals in guard cells are the main controllers of stomatal behavior of the plants (Mansfield et al. 1990). However, high concentration of soil heavy metals interferes badly on the calcium uptake of plants as stated by Ernst (1992). Therefore, this negative impact of soil heavy metals on calcium uptake may be the reason for disruption of the water balance in plant tissues. According to the present experiment, the only parameter that was affected significantly by the Cd treatments was Tr. Though, Tr get affected severely by variety Cd interaction effect, leaf RWC and stomatal conductance to water were remain unchanged. Therefore, increase of Tr was affected badly on leaf water content. Such type of recovery mechanism can be happening in plants due to biosynthesis of phytochelatins as tolerant agents (Cobbett and Goldsbrough 2002).

It has been documented that Cd damages the enzyme system where most of the cellular functions are mediated by transferring substances and information between cell and cell external environment. Hence, it increases the cell membrane permeability as stated by Hong et al. (1991) Li et al. (1992) and Azevedo et al. (2007). Further, the accumulation of ROS and malondialdehyde significantly increases under high Cd concentrations (Hatata and Abdel-Alal 2008). Thus, it increases the electrolyte leakage out of the leaf cells due to stimulated lipid peroxidation of cell membranes (Luo 1999: cited by Cheng 2003). Though the tested levels of Cd do not affect significantly on cell membrane permeability of rice leaves, a slight increase of total electrolytes was resulted in 3 mg kg^{-1} Cd treatment than its control in both rice varieties. This may be due to the cell membrane damage exerted by external Cd in the treatments.

4. Conclusions

The present experiment showed that the toxicity effect of Cd on shoot dry weight, photosynthetic rate and transpiration rate. All the other parameters including leaf area, root dry weight, death leaf percentage, relative leaf chlorophyll content, stomatal conductance to H_2O, intercellular CO_2 concentration, computed leaf temperature, cell membrane permeability and relative water content were not significantly affected by 0.5 and 3 mg kg^{-1} Cd concentrations. Bg300 slightly accelerated the plant growth when growing medium Cd was changed from 0.5 - 3 mg kg^{-1}. Therefore, the tested two rice varieties can compensate the toxic effect of 3 mg kg^{-1} Cd level in most of the parameters. Since the shoot growth was inhibited by Cd than the root growth, the root growth is not necessarily more sensitive than the shoot growth for the tested Cd levels. However, rice variety and Cd interaction was only changed the transpiration rate. Therefore, differential sensitivities of Bg300 and *Suwandel* cannot be distinguished under 0 – 3 mg kg^{-1} Cd levels. These results indicate that the both rice varieties can be grown successfully in 0 – 3 mg kg^{-1} Cd containing sites without experiencing significant impacts on their growth and physiology. However, further studies are necessary to validate these findings under various soil and environmental conditions representing various agro-ecological zones.

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Conflicts of Interest: The authors declare that there are no conflicts of interest regarding the publication of this paper.

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