

CHARACTERIZATION OF ROOT SYSTEM ARCHITECTURE UNDER ARSENIC AND CADMIUM STRESSES IN RICE

Rishiraj Raghuvanshi^{1,2}, Ashish Kumar Srivastava^{2,3*}, Satish Verulkar¹ and Penna Suprasanna³

¹Department of Plant Molecular Biology and Biotechnology, Indira Gandhi Krishi Vishwavidyalaya, Raipur-492012, India;

²Nuclear Agriculture and Biotechnology Division, Bhabha Atomic Research Centre, Mumbai-400085, India;

³Homi Bhabha National Institute, Mumbai-400094, India
Email: ashishbarc@gmail.com

Received-04.04.2021, Revised-20.04.2021, Accepted-28.04.2021

Abstract: Rice is major food crop for the world. Arsenic (As) cadmium (Cd) are the major heavy metal pollutant present in soil and water that disturb physiological and metabolic process of the plant cell and reduces the plant growth and yield. We have evaluated the effect of these two heavy metals on root system architecture in basmati and non-basmati (IR-64) rice genotypes. Both the variety showed significant increase in root length and number of lateral roots in MS medium at variable concentration (20, 40 and 60 μM) of As and Cd stresses, similarly root fresh weight, root dry weight and root length was found to be increased in soil medium under both the stresses condition. There was no significant difference was observed between IR-64 and Pusa basmati in root growth parameters.

Keywords: Arsenic, Cadmium, Heavy metal stress, Root system architecture

INTRODUCTION

Rice is the most important staple food in the world. Arsenic (As) and cadmium (Cd) are the non-essential toxic elements present everywhere in the environments. Anthropogenic activities are increasing every year due to industrial waste, municipal waste, excess application of pesticides and inorganic fertilizer have resulted increased heavy metal pollution in water and soil. As and Cd are toxic for all living organism (Kavamura and Esposito, 2010; Miransari, 2011). Paddy field is usually rich in As and Cd concentrations (Satpathy et al., 2014). Rice can able to accumulate 10 to 20 times more As than wheat and barley (Nunes et al., 2017). As and Cd both interferes in plant physiological process such as, carbohydrate metabolism, photosynthetic function, triggers the oxidative stress (ROS), nitric oxide (Wu et al., 2013; Singh et al., 2016) that lead to reduced plant growth and poor productivity. Presence of heavy metal such as As and Cd in soil and water affect the root system architecture and as well as the plant growth (Srivastava et al., 2021; Hussain et al., 2020). Root growth and development is a combination of cell division and cell elongation. Plant develop its root system by formation of lateral roots along the length of the primary root. As these lateral roots grow, they also give rise to new lateral roots. In present study, root growth parameters were evaluated under the As and Cd stress conditions to

assess the toxicity effect on root-system architecture in basmati and non-basmati (IR-64) rice varieties.

MATERIALS AND METHODS

Root phenotyping in agar plate

Rice seeds of IR-64 (Non-basmati research variety) and Pusa basmati-1 (widely cultivated aromatic variety) were surface sterilized by treating it with 70% ethanol for 10 min followed by 0.1% HgCl_2 for 10 minutes. The seeds were washed with sterile distilled water for more than five times to remove excess of HgCl_2 . The seeds were dried with sterile tissue paper. Dried seeds were then transferred on sterile Murashige and Skoog (MS) medium containing 0.8% agar. The plates were kept for germination in dark at 30°C for 3 days. Germinated seeds were transferred to fresh agar plates (MS media) containing variable concentration (20, 40 and 60 μM) of As (sodium arsenate) and Cd (cadmium sulfate). After one week of treatment, number of lateral root and root length were quantified.

Root phenotyping in soil medium

Rice seedlings were grown in hydroponics for 15 days then single plant was transferred into a polythene bag with three replications. Basic fertilizer dose was added as follows: Nitrogen (N) in the form of urea; 76.3mg/Kg⁻¹ soil (equivalent to 160 Kg/ha⁻¹) in four equal doses of 21mg at 30 days of interval.

*Corresponding Author

Phosphorus (P) in the form of $\text{Ca}(\text{H}_2\text{PO}_4)_2 \cdot \text{H}_2\text{O}$; 28.6 mg/Kg^{-1} soil (equivalent to 60 Kg/ha^{-1}) and potassium (K) in the form of KCl; 28.6 mg/Kg^{-1} soil (equivalent to 60 Kg/ha^{-1}) at the time of soil filling (Abedin et al., 2002). Seedling were subjected to As 50 mg/Kg^{-1} soil (sodium arsenate) and Cd 5 mg/Kg^{-1} soil (cadmium sulphate). Submerged condition was maintained in each polybag till harvesting. Root traits were recorded at maturity stage in term of root length, fresh weight and dry weight.

Statistical analysis

One-way ANOVA was performed, followed by Duncan's Multiple Range test (DMRT) based post-hoc ranking ($P < 0.05$) in SPSS 16 and graph was plotted by Graph pad prism.

RESULTS AND DISCUSSION

As and Cd are the most toxic heavy metal, present everywhere in the environment. These two heavy metals easily absorbed by the plant via nutrient transporters and disturb the physiological and metabolic function of the plants that lead to reduced plant growth and poor yield. In present study, root-system architecture (RSA) was evaluated under the As and Cd stress condition to assess the toxicity effect on root system with the help of two different experiments. Firstly, RSA study was done for IR-64 and Pusa-basanti-1 rice variety in agar plates at variable concentration (20, 40 and $60 \mu\text{M}$) of As and Cd stress and both the variety showed differential response in terms of root length and number of lateral root. Root length was found to be increased under both the stresses conditions. Both the variety showed higher root length at $20 \mu\text{M}$ of As stress (average: 7.34 cm for IR-64 and 9.4 cm for PB-1) and Cd stress (average: 7.5 cm for IR-64 and 9.9 cm for PB-1) followed by 40 and $60 \mu\text{M}$ of stresses (fig. 1, 2). Similarly, pot experiment also showed increase in root length for both the variety (fig. 3) under As (average: 36.5 cm for IR-64 and 37.5 cm for PB-1) as well as under Cd stress conditions (average: 37 cm for IR-64 and 37.5 cm for PB-1).

Plant develops its root system by formation of lateral roots along the length of the primary root. As these lateral roots grow, they also give rise to new lateral roots. The number and distribution of lateral roots are strongly affected by several environmental conditions (Casimiro et al., 2003). Thus, we have

analyzed the number of lateral root at variable concentration (20, 40 and $60 \mu\text{M}$) of As and Cd stresses. Number of lateral root was found to be increased in IR-64 at $20 \mu\text{M}$ (average: 76) while reduced at higher doses 40 (average: 60) and $60 \mu\text{M}$ (average: 20) of As stress while Cd stress showed higher number of lateral root at $40 \mu\text{M}$ (average: 74) than 20 and $60 \mu\text{M}$ (fig. 1). Similarly, PB-1 showed significant increase in number of lateral root at $20 \mu\text{M}$ (average: 158) followed by 60 (average: 115) and $40 \mu\text{M}$ (average: 111) of As stress while dose dependent decreases (Average: 142, 118, 106) was seen in case of Cd stress condition (fig. 2).

Root biomass is an important trait, affected by several abiotic stresses including heavy metals contamination. In present study, root fresh weight was found to be increased significantly by 44 % (IR-64) and 54 % (PB-1) under As stress, whereas 32 % (IR-64) and 45 % (PB-1) under Cd stress condition (fig. 3). Similarly, root dry weight was also found to be increased significantly by 42 % (IR-64) and 36 % (PB-1) under As stress, whereas 21 % (IR-64) and 43 % (PB-1) under Cd stress condition (fig. 3). The result obtained by both the experiment showed that As and Cd both the stresses significantly affect root system architecture by increasing root length and biomass. In contrast reduced shoot length and poor plant growth have been reported under both the stresses (Srivastava et al., 2021; Hussain et al., 2020; Yadav et al., 2021; Abedi et al., 2021). There is no specific transporter reported for As and Cd transport. These enter into the root tissue via nutrient transporters and alter root physiology by disturbing endogenous level of phyto-hormones (Ronzan et al., 2018). Both the stresses alter the auxin level and disturb its localization, result in cell proliferation in root tissue (Ronzan et al., 2018; Srivastava et al., 2021), hence increased root length and biomass was observed under both the stresses. Auxin is the key plant hormone for most of the plant physiological activities and particularly important for root meristem organization, with its action made possible by the realization of an IAA gradient, involving coordination between its synthesis and polar transport (Blilou et al., 2005). Thus biosynthesis of auxin and its proper distribution to different organ is required for normal growth and development.

Figures legend

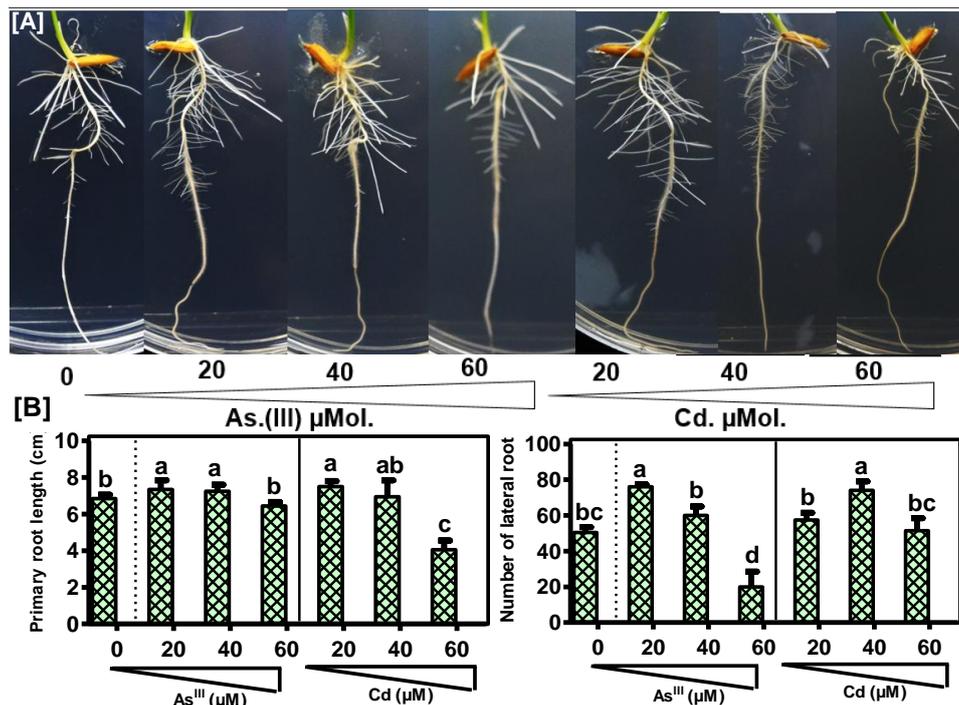


Figure 1: Evaluation of root traits of IR-64 under As and Cd stress condition. Germinated seeds of IR-64 were transferred to fresh agar plates (MS media) containing variable concentration (20, 40 and 60 μM) of As (sodium arsenate) and Cd (cadmium sulfate). After one week of treatment, differentials phenotypes

were recorded qualitatively (A) and quantitatively (B) in terms of root length (cm) and number of lateral roots. Different letters on the bar graphs have been placed on the basis of the LSD values derived from SPSS software (DMRT, $p \leq 0.05$).

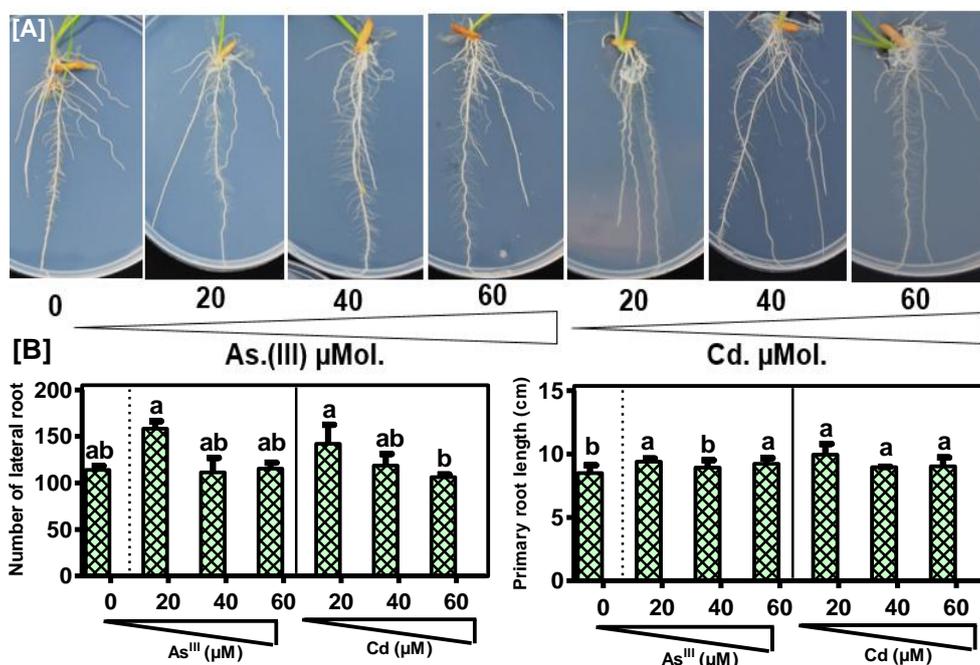


Figure 2: Evaluation of root traits of Pusa-basmati-1 under As and Cd stress condition. Germinated seeds of PB-1 were transferred to fresh agar plates (MS media) containing variable concentration (20, 40 and 60 μM) of As (sodium arsenate) and Cd (cadmium sulfate). After one week

of treatment, differentials phenotypes were recorded qualitatively (A) and quantitatively (B) in terms of root length (cm) and number of lateral roots. Different letters on the bar graphs have been placed on the basis of the LSD values derived from SPSS software (DMRT, $p \leq 0.05$).

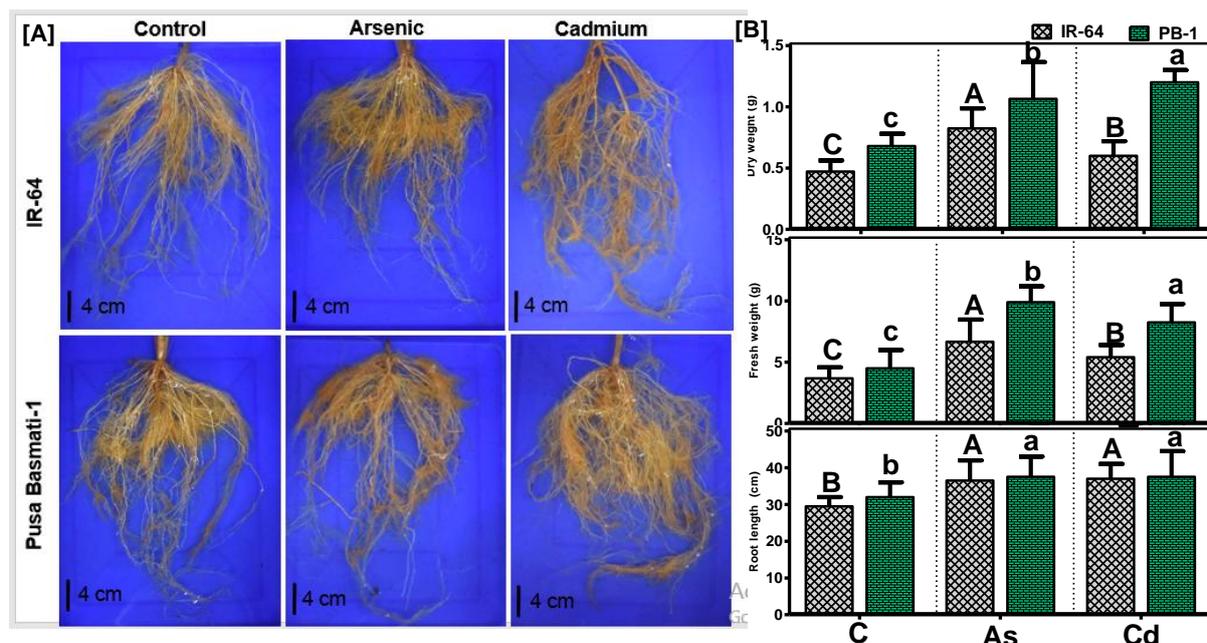


Figure 3: Root phenotyping of rice in soil medium under As and Cd stress condition. The 15-d old rice seedlings (variety IR-64 and PB-1) were independently transplanted to pots and subjected to sodium arsenate (50mg/Kg^{-1} soil) and cadmium sulphate (5 mg/Kg^{-1}) stresses. Basic fertilizer dose was added in each plants. Differential phenotypes were recorded qualitatively (A) and quantitatively (B) in terms of root length (cm) and root fresh weight and root dry weight. Different letters on the bar graphs have been placed on the basis of the LSD values derived from SPSS software (DMRT, $p \leq 0.05$).

CONCLUSION

The plant root system affected by several environmental factors. As and Cd stresses significantly effect the root system architecture by disturbing endogenous hormones level. As and Cd both significantly increased root length, number lateral roots and root biomass and hence increase energy expense during these stresses which is required for plant growth and developments. However, stress response is varying with genotypes. We evaluated basmati and non-basmati rice, both the variety showed almost similar response in terms of root system architecture.

REFERENCES

Abedi, E. (2021). Cadmium stress in rice plants: The effect of cadmium on seed germination and seedling growth of rice plant (*Oryza sativa* L.). *The Open Access Journal of Science and Technology*, 9(1), 5-7.
Blilou, I.; Xu, J.; Wildwater, M.; Willemsen, V.; Paponov, I.; Friml, J. and Scheres, B. (2005). The PIN auxin efflux facilitator network controls growth

and patterning in *Arabidopsis* roots. *Nature*, 433 (7021), 39-44.

Casimiro, I.; Beeckman, T.; Graham, N.; Bhalerao, R.; Zhang, H.; Casero, P. and Bennett, M. J. (2003). Dissecting *Arabidopsis* lateral root development. *Trends in plant science*, 8(4), 165-171.

Hossain, M. A.; Piyatida, P.; da Silva, J. A. T. and Fujita, M. (2012). Molecular mechanism of heavy metal toxicity and tolerance in plants: central role of glutathione in detoxification of reactive oxygen species and methylglyoxal and in heavy metal chelation. *J. Bot.* 2012:872875. doi: 10.1155/2012/872875.

Hussain, B.; Ashraf, M. N.; Abbas, A.; Li, J. and Farooq, M. (2020). Cadmium stress in paddy fields: Effects of soil conditions and remediation strategies. *Science of The Total Environment*, 142188.

Kavamura, V. N. and Esposito, E. (2010). Biotechnological strategies applied to the decontamination of soils polluted with heavy metals. *Biotechnology advances*, 28(1), 61-69.

Miransari, M. (2011). Hyperaccumulators, arbuscular mycorrhizal fungi and stress of heavy metals. *Biotechnology Advances*, 29(6), 645-653.

Nunes, L. M. and Otero, X. (2017). Quantification of health risks in Ecuadorian population due to dietary ingestion of arsenic in rice. *Environmental Science and Pollution Research*, 24(35), 27457-27468.

Ronzan, M.; Piacentini, D.; Fattorini, L.; Della Rovere, F.; Eiche, E.; Riemann, M. and Falasca, G. (2018) Cadmium and arsenic affect root development in *Oryza sativa* L. negatively interacting with auxin. *Environmental and Experimental Botany*, 151, 64-75.

Satpathy, D.; Reddy, M. V. and Dhal, S. P. (2014). Risk assessment of heavy metals contamination in paddy soil, plants, and grains (*Oryza sativa* L.) at the

East Coast of India. *Bio Med research international*, 2014.

Singh, S.; Parihar, P.; Singh, R.; Singh, V. P. and Prasad, S. M. (2016). Heavy metal tolerance in plants: role of transcriptomics, proteomics, metabolomics, and ionomics. *Frontiers in plant science*, 6, 1143.

Srivastava, A. K.; Pandey, M.; Ghate, T.; Kumar, V.; Upadhyay, M. K.; Majumdar, A. and Suprasanna, P. (2021). Chemical intervention for enhancing growth and reducing grain arsenic accumulation in rice. *Environmental Pollution*, 276, 116719.

Sui, F. Q.; Chang, J. D.; Tang, Z.; Liu, W. J.; Huang, X. Y. and Zhao, F. J. (2018). Nramp5

expression and functionality likely explain higher cadmium uptake in rice than in wheat and maize. *Plant and Soil*, 433(1), 377-389.

Wu, F.; Fang, Q.; Yan, S.; Pan, L.; Tang, X. and Ye, W. (2020). Effects of zinc oxide nanoparticles on arsenic stress in rice (*Oryza sativa* L.): germination, early growth, and arsenic uptake. *Environmental Science and Pollution Research*, 27, 26974-26981.

Yadav, P.; Srivastava, S.; Patil, T.; Raghuvanshi, R.; Srivastava, A. K. and Suprasanna, P. (2021). Tracking the time-dependent and tissue-specific processes of arsenic accumulation and stress responses in rice (*Oryza sativa* L.). *Journal of Hazardous Materials*, 406, 124307.

