Effect of plant growth regulators on rice plants (*Oryza sativa* L.) growth under cadmium stress

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Abstract. In numerous developing countries, including Kazakhstan, the issue of soil contamination with cadmium is prominent due to the expansion of the mining and metallurgical sectors. A significant contributor to cadmium pollution in soil is the widespread application of phosphorus-based fertilizers and pesticides that contain cadmium. Even trace amounts of cadmium, accumulating in the soil, can decrease crop yields, impede plant growth, and disrupt various physiological and biochemical processes. Consequently, this study aimed to identify cadmium-resistant rice varieties and explore methods to mitigate cadmium toxicity using growth regulators. The investigation examined the effects of growth-stimulating substances, such as "Epin-Extra" (an epibrassinolide alcohol solution), "Cyrcon" (a hydroxycinnamic acid solution), and "Beres-4 universal" (potassium humate), under cadmium-induced stress. Additionally, the research aimed to elucidate how the structural components of these substances relate to reducing cadmium's toxic impact on rice plants. The findings suggest that these growth-stimulating substances could be beneficial in ameliorating the adverse effects of cadmium on plants.

1 Introduction

Cadmium (Cd), a heavy metal, exerts a detrimental impact on the biochemical and physiological functions of plants, leading to a suppression of their growth and development. An elevation in cadmium concentration within soil at the plant roots results in stunted growth, a reduction in root length, a decrease in the number of lateral roots, the death of root hairs, and a reduction in biomass [1-3]. The issue of soil pollution with cadmium is increasingly pressing due to the application of cadmium-containing phosphorus fertilizers and pesticides [4, 5]. This results in Cd accumulation in soil, adversely affecting crop yield and development by disrupting physiological and biochemical processes in plants.

Cd's influence on cell division and elongation directly corresponds to the observed hindrance in plant and root growth. According to various studies, the rate of cell division in plants decelerates, the quantity of cells undergoing mitosis diminishes, the duration of mitotic phases extends, and the entire mitotic cycle is prolonged. These alterations are attributed to Cd's strong binding to the sulfhydryl groups of spindle proteins and mitosis-regulating enzymes. Cd presence leads to reduced elongation growth due to diminished cell wall elasticity, damage to microtubule structure, altered cell water regulation, and membrane permeability. Such disturbances result in decreased nutrient and water absorption, negatively impacting plant growth, development, and productivity.

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Elevated Cd levels in plants' environment cause reductions in stem size, internode number, both wet and dry biomass, inflorescence size, as well as leaf size, leading to diminished photosynthesis and transpiration rates [6-8].

Photosynthesis is notably susceptible to Cd exposure. Leaf chlorosis represents a symptom of Cd toxicity affecting the photosynthetic machinery, also influencing the concentration of green pigments. The inhibition of chlorophyll production, chloroplast ultrastructure disruption, iron deficiency, and altered mineral nutrition all contribute to the reduced green pigment levels in leaves due to Cd. Moreover, Cd negatively impacts photosynthesis by decreasing carotenoid levels in susceptible plants [9, 10].

Plant growth regulators (PGRs), commercially utilized chemicals in agriculture for disease control, growth stimulation, and yield enhancement, include various phytohormones, jasmonates, brassinosteroids (BR), humic substances (HS), among others. HS, in particular, can bind metal ions, including toxic pollutants, forming complexes that may lessen soil metal toxicity. They induce a range of morphological, physiological, and biochemical effects on plants [11].

HS are crucial elements of terrestrial ecosystems, renowned for their capability to bind with metal ions, and both mineral and organic compounds, including toxic pollutants, to form complexes that potentially mitigate soil metal toxicity. Research has demonstrated that HS promotes the growth of roots, leaves, and shoots, and stimulates germination across various crop types. These benefits are attributed to HS's interaction with plant physiological and metabolic processes, enhancing nutrient uptake, cell permeability, and growth stimulation mechanisms [12, 13]. Consequently, this study aims to explore the impact of PGRs on mitigating Cd toxicity in different rice varieties to reduce the adverse effects of this pollutant.

2 Materials and Methods

This study investigated the influence of Cd and plant growth regulators (PGRs) on four varieties of rice: Kazvetta, Aysaule, KazNIIR5, and KazEr6. The rice seedlings were cultivated in hydroponic conditions within plastic containers in 3 replicates each variant. For the experiments, 150 μM $CdSO_4$ and various PGRs were utilized, including "Epin-Extra" (an epibrassinolide solution in alcohol with a 0.025 g/L concentration), "Cyrcon" (a solution of hydroxycinnamic acids, 0.05 ml/100 ml H_2O), and "Beres-4 universal" (Potassium humate, 40%). These agents were applied through two methods: seed soaking and addition to the nutrient medium. The evaluation of biometric parameters followed established methods. Plants were separated into aboveground and belowground parts for measurement of organ lengths. To ascertain the dry biomass of roots and aboveground parts, samples underwent drying at 105°C until reaching a consistent weight, followed by cooling to ambient temperature for weighing. The seeds were soaked in solutions of these growth regulators and added to the growth medium according to the attached instructions. In the soaking variants, rice seeds were soaked in a solution of 10 μl per 100 ml of water for 60 seeds, the exposure time was 18-20 hours, according to the manufacturer's prescription. In variants with the addition of growth regulators to the medium, the following concentrations were used: "Cyrcon" - 10 μl per 100 ml; "Epin-Extra" - 50 μl per 100 ml; "Potassium humate" - 10 μl per 100 ml. Statistical data processing was carried out using two-way analysis of variance with varieties and treatments as the main factors and one-way variance analysis to determine the significance of the difference between control and treatments using the Statistica 11.5 package and Excel 2010 from the Microsoft Office XP package.

3 Results and discussion

The study examined the impact of plant growth regulators (PGRs) on the growth metrics of 10-days rice seedlings grown in Cd-free growth medium. Figure 1 shows that all tested PGRs significantly enhanced the linear growth of the aboveground parts of the plants. However, the extent of positive growth varied across different rice varieties. Notably, "Potassium humate" showed the most substantial improvement in the shoot lengths of the Aysaule and Kazvetta varieties, with increases of 44% and 58% for seed soaking, and 24% and 29% when added to the growth medium, respectively, compared to the control group.

Investigating the influence of plant growth regulators (PGRs) on the accumulation of biomass in the aboveground parts of plants in a Cd-free medium revealed that "Cyrcon" and Figure 2 demonstrates that "Potassium humate" had a more beneficial impact compared to "Epin-Extra".

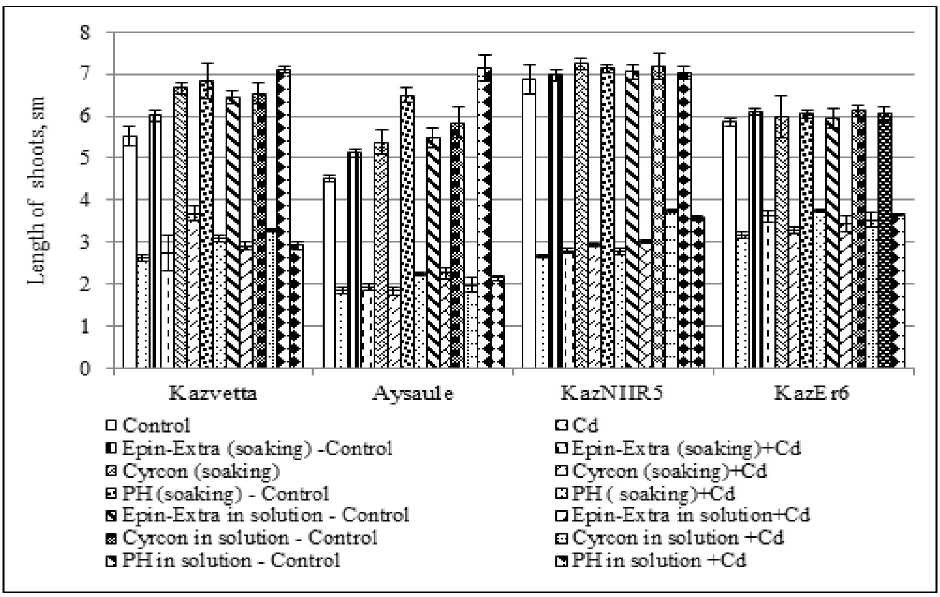

Fig.1. The effect of Cd and PGR on the linear growth of aboveground organs in 10-days rice seedlings ($p < 0,05$).

The study also assessed the impact of PGRs on shoot biomass in medium containing Cd, finding that all tested PGRs mitigated Cd's adverse effects across all varieties to varying extents. "Potassium humate" was particularly effective in reducing Cd toxicity, with the most notable improvement seen in the KazNIIR5 variety when added to the growth medium, showing a 19% decrease in Cd's negative impact (Figure 2).

Regarding the influence of Cd on shoot biomass, the most considerable reduction occurred in the KazNIIR5 variety (77%), while the Aysaule variety showed a lesser decrease (47%). Other varieties exhibited intermediate levels of impact. Resistance to biomass reduction under Cd exposure ranked as follows: Aysaule (53%) > KazEr6 (47%) > Kazvetta (28%) > KazNIIR5 (23%). The most stimulating effects of PGRs on shoot biomass were observed in the KazEr6, Kazvetta, and Aysaule varieties with specific PGRs, indicating a variety-dependent response to PGRs on growth (Figure 2).

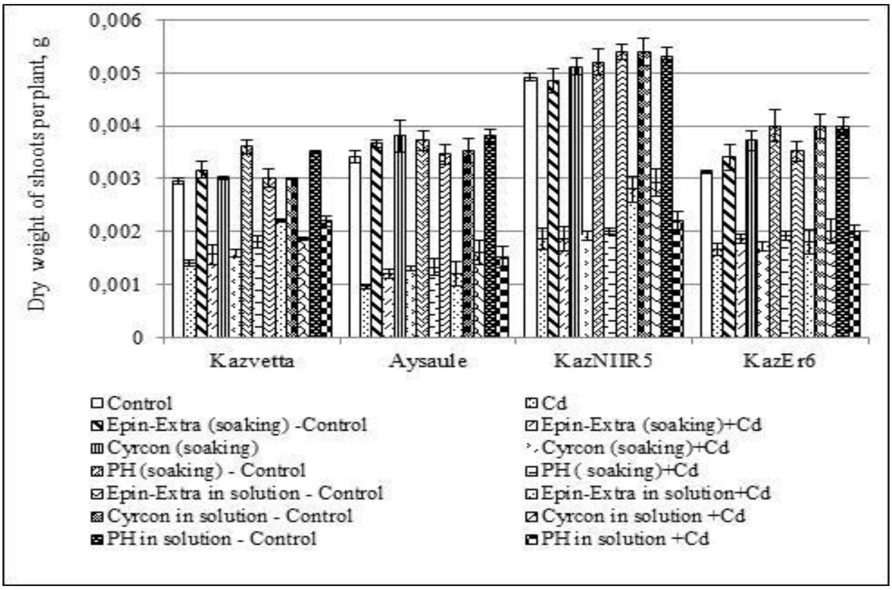

Fig. 2. The effect of Cd and PGRs on biomass accumulation by aboveground organs of 10-days rice seedlings ($p < 0,05$).

In terms of resistance to Cd based on the length of aboveground organs, the varieties ranked as follows, with percentages relative to control in the presence of 150 µM Cd: KazEr6 (54%) > Kazvetta (48%) > Aysaule (40%) ≥ KazNIIR5 (39%).

Examining the effects of PGRs on linear root growth in control (Cd-free) conditions, "Potassium humate" stood out for its positive impact, especially in the Kazvetta variety, where root length increased by 59% over the control (Figure 3).

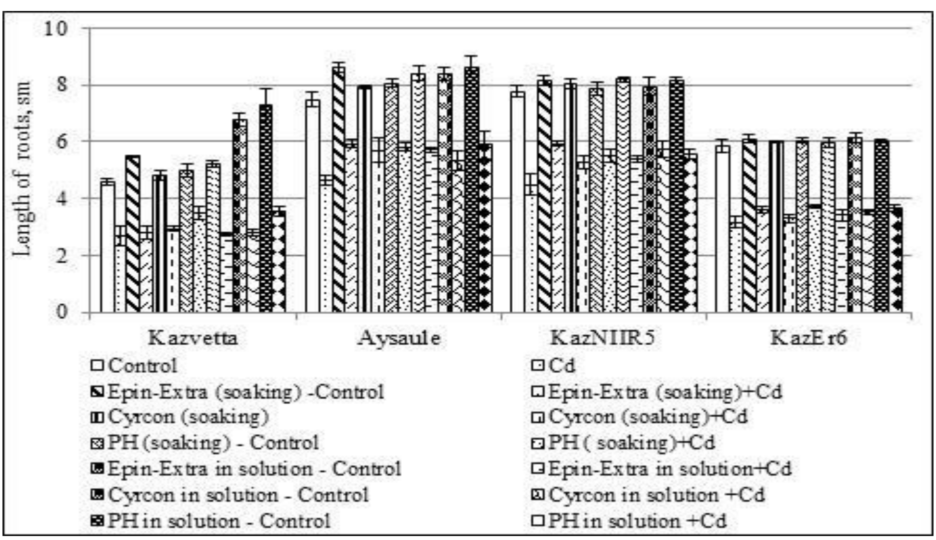

Fig. 3. The effect of Cd and PGRs on the linear roots growth of 10-days rice seedlings ($p < 0,05$).

The Kazvetta and Aysaule varieties also experienced growth increases with other PGRs, albeit to a lesser degree. The KazNIIR5 and KazEr6 varieties showed minimal susceptibility to PGRs, with root growth reaching 104-105% of the control, underscoring the specific response of different varieties to PGR treatment.

Cd significantly hindered linear root growth in the studied rice varieties, with the Aysaule variety experiencing the least suppression (38%) and the KazEr6 variety the most (46%). The application of plant growth regulators (PGRs) was found to mitigate Cd's inhibitory effect on root growth to various extents, depending on the rice variety and the method of PGR application. Among the PGRs tested, "Potassium humate" consistently demonstrated the most positive impact on root length across most varieties, whether applied through soaking or added to the growth medium (Figure 3).

Cd exposure also markedly reduced root biomass accumulation compared to aboveground parts. The KazNIIR5 variety was the most adversely affected, with a 77% reduction in root dry biomass, while the KazEr6 variety showed a somewhat lesser decrease (64%). The resistance to biomass reduction by roots in the presence of 150 µM Cd was ranked as follows: KazEr6 (34%) > Aysaule (24%) = Kazvetta (24%) ≥ KazNIIR5 (23%), highlighting the variability in Cd sensitivity among the varieties (Figure 4).

The research revealed that the application of plant growth regulators (PGRs) in a Cd-free medium modestly enhanced root biomass. Among the PGRs examined, "Potassium humate" was the most effective, boosting root biomass by 4 to 17% across different rice varieties (Figure 4).

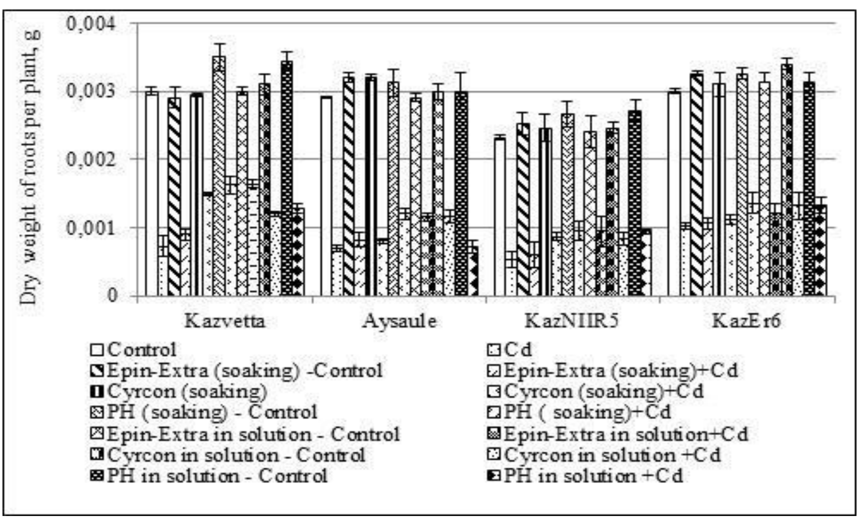

Fig. 4. The effect of Cd and PGRs on roots biomass of 10-days rice seedlings (p < 0,05).

The application of PGRs enhanced root biomass accumulation in rice plants relative to conditions with Cd present in the growing medium. Specifically, "Potassium humate" and "Cyrcon" demonstrated the most substantial positive impacts on root biomass, particularly when seeds were soaked in these solutions. While adding these substances directly to the growing medium did not reveal a consistent pattern in mitigating Cd's toxic effects, it was observed that all examined PGRs to some extent alleviated the inhibitory impact on root biomass. This suggests that, despite the variability in response depending on the application method, PGRs overall contribute to reducing the detrimental effects of Cd on root biomass accumulation.

Conclusion

The research on the effects of Cd on the growth parameters of different rice varieties indicated that Aysaule and KazEr6 varieties exhibit relative tolerance to cadmium stress, whereas the KazNIIR5 variety is more sensitive, with Kazvetta falling in an intermediate category. In scenarios devoid of Cd, the application of "Epin-Extra", "Cyrcon", and "Potassium humate" through various methods, including seed soaking and solution addition, led to enhanced growth rates. In the presence of Cd, "Potassium Humate" and "Cyrcon" emerged as the most effective growth regulators across most rice varieties.

The advantageous impact of “Potassium humate” may be attributed to its comprehensive role in enhancing nitrogen and carbon metabolism, influencing physiological processes through gene expression modulation, and participating in the signaling pathway. The positive influence of "Epin-Extra" is likely due to the brassinosteroids in its formulation, known for promoting cell division and elongation. Meanwhile, the beneficial effect of "Cyrcon" under Cd exposure is linked to its hydroxycinnamic acid content, a type of phenolic compound recognized for its antioxidant and protective properties. Based on further experiments, "Potassium humate" and "Cyrcon" are suggested for use under adverse environmental conditions to alleviate the toxic impact of pollutants.

Authors' contribution

Conceptualization - Atabayeva S.D.; Methodology – Rakhymgozhina A.B., .Shoinbekova S.A.; Investigation - Asrandina S.Sh, Doktyrbay G.; Writing – Original Draft Preparation-Rakhymgozhina A.B.; Writing – Review & Editing - Atabayeva S.D.

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