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COPING THE ARSENIC TOXICITY IN RICE PLANT WITH MAGNESIUM ADDENDUM FOR ALLUVIAL SOIL OF INDO-GANGETIC BENGAL, INDIA

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Highlights

It has been experimentally found that:

- By application of Mg-salt as fertilizer As-induced toxicity can be reduced.
- In As contaminated soil As has got competitive advantage over Mg but application of Mg fertilizer is able to mitigate the problem.
- Mg is an important plant nutrient hence there is no extra cost involved and it is an eco-friendly & economic method.

Abstract. Arsenic (As^{3+}) is a toxic metalloid found in the earth's crust, its elevated concentration is a concern for human health because rice is the staple grain in eastern part of India and the waterlogged rice field environment provides opportunity for more As^{3+} uptake. Magnesium (Mg^{2+}) is an important plant nutrient. Present work is a search for reducing As^{3+} toxicity in plants through Mg^{2+} application. The findings are quite impressive, the root to shoot biomass ratio showed more than 1.5 times increase compared to the control. Total protein content increased 2 folds. Carbohydrate and chlorophyll content increased two to three times compared to control. On the other hand, Malondialdehyde content showed a decline with the application of increased Mg^{2+} dose. The *in-silico* study shows a better interaction with As^{3+} in presence of Mg^{2+} but interestingly without stress symptoms. These findings from the research indicate that Mg^{2+} application can be effective in reducing As^{3+} induced stress in plants.

Keywords: toxicity, *in-silico* study, oxidative stress, cation competition, environmental sustainability, waste management technologies.

Introduction

Arsenic (As^{3+} and As^{5+}) is a ubiquitous metalloid found in earth's crust. There is gradual increase in soil As^{3+} level due to different anthropogenic activities (Chandrakar et al., 2016). It is a non-essential element for plants but depending on the concentration gradient between the source and the sink it is taken up from soil mostly in inorganic forms with the help of various transporter proteins. Among the two oxidation states of As^{3+} and As^{5+}, the As^{5+} form is less toxic and is found in immobile mineral form (Shrivastava et al., 2015). The inorganic form of arsenic is more toxic than the organic form.

Sources of As^{3+} poisoning include agricultural land and urban areas, in countries around the world of which Bangladesh, Pakistan, Nepal, Vietnam, Burma, Thailand and Cambodia are the most affected. Hence As^{3+} contamination in the environment is considered as a global problem. As^{3+} stress can affect the growth and productivity of the plants by a plethora of (Singh et al., 2017) physiological and biochemical alterations and the most damaging one is the production of reactive oxygen species (ROS). Plants sometimes develop various tolerance and adaptive mechanisms to cope with stresses which involve a series of physiological and biochemical changes (Khalid et al., 2017). As^{3+} is also reported to inhibit rate of photosynthesis in plants.

Rice (*Oriza sativa* L) is a potentially important route of human exposure to arsenic as staple food of the people in tropical, subtropical and temperate regions of East Asia. The As^{3+} in soil can be accumulated by rice plants and can reach to human being through food intake. It has been highlighted that the rice plants are more susceptible for As^{3+} accumulation because of changing in redox

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condition during flooding condition in the paddy fields (Ma, 2001). In case of cultivation in a large area with moderately As^{3+} infested soil it is difficult to remove soil As^{3+}. Phytoremediation is practised as an eco-friendly and the most economic remediation technique. This technique is dependent on many environmental parameters and under best possible conditions (Chaudhry et al., 2002) also the efficiency is limited. Another major concern for phytoremediation is the proper disposal of the huge contaminated biomass generated.

Mg^{2+} is the second most important nutrient for the plants and is part of large numbers of metabolic pathways. According to Cakmak and Yazici (2010), Chloroplast in plants contains 31% of the total Mg^{2+} content; it is required for chlorophyll formation and plays a key role in photosynthetic activity. Wingler et al. (2005) explained that under Mg-deprivation due to a reduction in electron transport, chlorosis takes place in plants, which impairs CO_2 fixation and induces generation of Reactive Oxygen species (ROS). These can cause damage of cell components, like membrane lipids, proteins, and nucleic acids, resulting in metabolic disruption (Scandalios, 2005). An increased activity of antioxidant defense enzymes has been reported in some plant species under Mg-deficient conditions (Tewari et al., 2005; Shulaev et al., 2008).

The importance of Mg^{2+} in crop production was well estimated only in the last decade (Cakmak & Yazici, 2010), agronomists and scientists have paid little attention to this mineral nutrient compared to others. There are prominent reasons for Mg^{2+} deficiency to occur in plants, among them, the effects of cation competition (Ca^{2+}, K^+ and Na^+) were found to be significant (Broadley & White, 2010) which is further accelerated with addition of 'N-P-K' fertilizer without adding Mg^{2+}. Hence in the cultivation process without using Mg^{2+} fertilizer, there is enough reason for Mg^{2+} deficiency in the plants.

From the above discussions it is understandable that soil As^{3+} in plants adversely affects various metabolic processes which are manifested as stress symptoms in plants. Mg^{2+} regulates a lot of metabolic activities in plants and its deficiency also causes stress symptoms. All these factors corroborate the scientific fact that Mg^{2+} in plants can be helpful in reducing physiological and biochemical problems. In addition, Mg^{2+} uptake by plants gets adversely affected due to cation competition and it indicates requirement of additional magnesium fertilizer in the cultivation process.

Under such condition the objectives of this present study are to (i) understand the most effective role of soil Mg^{2+} fertilizer in reducing As induced stresses in rice plants, (ii) highlight the nature of transporter proteins involve in the competitive uptake (iii) propose an eco-friendly and economic process for remediation of As toxicity in rice plants.

1. Materials and method

1.1. Criteria of selection of Plant species

Rice being the staple consumed form of carbohydrate in Indo-Gangetic plains which is recognised as a potential source of As (As^{3+}) contamination from various studies (Ma, 2001). The rice plants are more susceptible to As (As^{3+}) contamination due to favourable changes of physico-chemical condition of soil during its growth and it can be the easiest way to human contamination through food consumption. Thus rice is chosen as the experimental plant in the present study. It is a monocot plant belongs to the Gramineae family and the genus Oryzae and the plant variety used for the experiment was *Shatabdi* (IET4786). Experimental set-up was done in September 2019 and harvesting was in January 2020 and the testing of parameters was done during January–February 2020.

1.2. Setting up of the experiment

The rice seed, collected from Rice Research Station, Chinsurah, West Bengal were allowed to germinate in a pot and 8–10 days old seedlings were first sown, then these were transplanted after another 15 days into a total of 12 different pots comprising of one control and three experimental sets, each in triplicate (Table 1). Each pot had 3 Kg of soil with a definite amount of magnesium (Mg^{2+} salt, magnesium carbonate) and Arsenic (As^{3+} salt, sodium arsenite) addendum in addition 2 gms of compost was added in each pot in divided dose as fertilizer for normal growth of the plants. The rice seedlings were added uniformly in a ratio of 10 seedlings per pot and the plants were grown till the panicle stage. All settings were done in triplicate and the results were represented as an average.

An *in-silico* study was performed to have idea on the transporter proteins involvement in the competitive transportation process. A molecular docking study was done to understand the protein-ligand interaction. Previous to the docking interaction studies, the 3D structure of the root transport protein of *Oryza sativa* (PDB ID 6OCE) was selected (Maity et al., 2019). The protein that was taken into consideration was a hypothetical transporter protein that was determined using the X-ray crystallographic studies.

Table 1. Showing experimental set-up: pots with different addendum

Sl No.	Pot marking	Addendum
1	C – Control	As^{3+} (30 mg/kg) without Mg^{2+}
2	Experimental-1 (E1)	Mg^{2+} (5.5 gm/kg) + As^{3+} (30 mg/kg)
3	Experimental-2 (E2)	Mg^{2+} (6.5 gm/kg) + As^{3+} (30 mg/kg)
4	Experimental-3 (E3)	Mg^{2+} (7.5 gm/kg) + As^{3+} (30 mg/kg)

1.3. Estimation of soil pH

The pH of soil sample was measured in soil solution with dilution ratio of soil: solution as 1:2.5. The samples were equilibrated to the normal temperature. The pH was measured by digital pH meter (model no. Systronics-802) after standardisation, soil pH was tested as 7(±0.2).

1.4. Estimation of malondialdehyde content

The sample extract was prepared by grinding the fresh plant sample followed by centrifugation and collecting the supernatant. In the supernatant 20% TCA (Trichloroacetic Acid) and 0.5% TBA (Thiobarbituric acid) were added and mixed well. The mixture was boiled and then quickly cooled on ice and then centrifuged. The supernatant was collected and the absorbance was recorded at 532 nm. The concentrations were calculated by graphical plotting against a standard curve using different concentrations of malondialdehyde (Zhang & Huang, 2013).

1.5. Estimation of total chlorophyll content

The fresh plant samples were ground in liquid nitrogen using acetone and supernatant was collected for preparation of the sample extract. This procedure was repeated till the residue becomes colourless. The absorbance was measured by colorimeter (Digital Photo Colorimeter, model No. LT-12, LABTRONICS) at 645 nm, 663 nm to calculate the chlorophyll concentration (Sadasivam & Manickam, 2008).

1.6. Estimation of total carbohydrate

The fresh samples were acid digested and then neutralized with sodium carbonate and centrifuged. To the supernatant anthrone was added, boiled, cooled and the absorbance (Digital Photo Colorimeter, model No. LT-12, LABTRONICS) recorded at 630 nm. The concentrations were calculated by graphical plotting against a standard curve using different concentrations of standard glucose (Sadasivam & Manickam, 2008).

1.7. Estimation of total protein

The fresh plant samples were ground followed by centrifugation and supernatant collected. To this extract alkaline copper solution was added and mixed well followed by addition of Folin – ciocalteau reagent, mixed well and incubated in the dark for 30 min. The absorbance was measured at 660 nm (Digital Photo Colorimeter, model No. LT-12, LABTRONICS). The concentrations were calculated by graphical plotting against a standard curve using different concentrations of bovine serum albumin (Sadasivam & Manickam, 2008).

1.8. Docking interaction study

Ligand dependent protein docking was performed using the Lamarckian Genetic Algorithm (LGA) method. Standard docking settings were applied and the energetically most favourable binding poses (lowest docked energy) were taken to obtain the best conformation. The prediction was done using Auto-Dock-Vina software. The docking interaction studies were performed between the transport protein, Arsenic (As^{3+}) and Magnesium (Mg^{2+}). The unwanted water molecules that were present in the protein was separated and the protein was stabilized at pH 7 (±0.2). The most stable conformation of the determined structures of the prepared ligand as predicted was selected to flexibly dock against the created receptor grid. The interaction was studied under "Ligand interaction diagram" and the types of interaction and bond lengths were predicted. Finally, the interaction of the metal with amino acid was performed using Ligplot.

1.9. Statistical significance

The experimental results were analyzed by using paired T test following standard statistical methods and the data were subjected to estimate significant level. Only the $p < 0.05$ or 0.01 was considered and were shown in the figures. Descriptive statistics including mean, average, standard deviation and standard error were considered to represent the precision of the analysis. All results showed more than 90% confidence level.

2. Results and discussion

In the present study, different biochemical plant parameters were assessed to understand the As^{3+} induced toxicity at cellular level in the rice plants and the role of Mg^{2+} in mitigating the same. Also through *in silico* study the interaction of the transporter protein with As^{3+} and Mg^{2+} was observed. It has been reported that As^{3+} has a negative impact on plant biomass. Hence to understand the effect of As^{3+} on the plant biomass and also the contribution of Mg^{2+} in it, the root to shoot biomass ratio was studied.

2.1. Comparison of plant root to shoot growth

In the experimental result as shown in Figure 1 the control plant with only As^{3+} dose and no Mg^{2+} the root to shoot biomass ratio was quite low, where as in the plants with

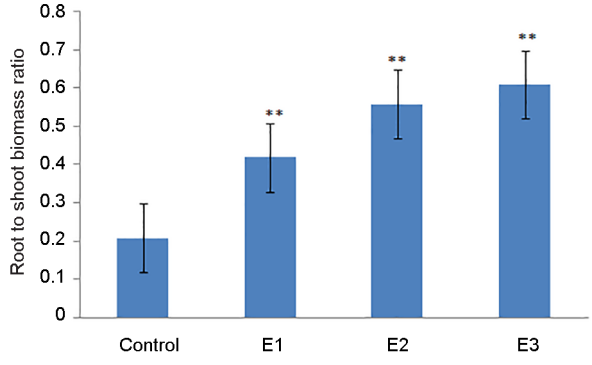


Figure 1. Comparison of changes in root to shoot biomass ratio in the experimental plants

application of different doses of Mg^{2+} the root to shoot biomass ratio was increased gradually with increase of Mg^{2+} dose. At higher concentration As^{3+} is toxic for almost all plants and reduces growth of both root and shoot, depresses tillering (Abedin et al., 2002). In many research works it has also been reported that Mg^{2+} deficiency in plants causes decrease in root to shoot biomass ratio (Cakmak et al., 1994a, 1994b; Mengutay et al., 2013).

In a study by Farhat et al. (2016) it was reported that magnesium fertilization increases total plant biomass by 61% compared to Mg^{2+} deficient control plants (Farhat et al., 2016). The positive effect of magnesium fertilization on the root biomass (77%) was greater than the shoot biomass (59%) as described by da Silva et al. (2014). The experimental results (Figure 2) also showed a considerable increase in root lengths with increased doses of Mg^{2+} fertilizer compared to the control plant with only As^{3+} and no Mg^{2+}.

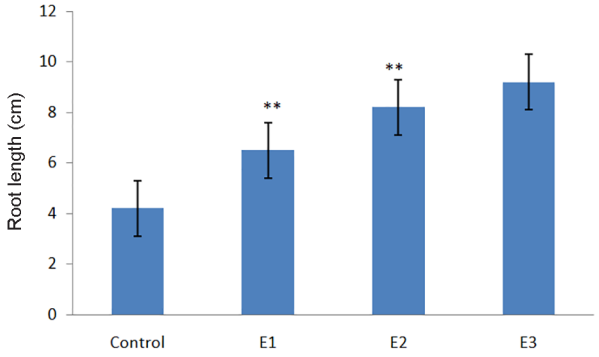


Figure 2. Comparison of root length between the experimental plants and control

2.2. Plant stress parameters

In general, exposure to As^{3+} reduces chlorophyll and protein content and photosynthetic activity in plants (Marin et al., 1993). In plants photosynthetic limitation results in reduced capacity for biochemical utilization of absorbed light energy which induces the formation of reactive oxygen species (ROS) such as superoxide radicals and hydrogen peroxide (Hauer-Jákli & Tränkner, 2019). In the present work the Mg^{2+} deficient and only As^{3+} exposed control plants showed less chlorophyll content (Figure 3) compared to the plants with Mg^{2+} application where total chlorophyll content had increased significantly.

It has been reported that Mg^{2+} directly affects the activity of Rubisco enzyme and activation by binding to the carbamylated Rubisco side chain (Hazra et al., 2015) and Mg^{2+} supply significantly enhances net photosynthetic CO_2 assimilation compared to the plants with Mg^{2+} deficiency. In the present study, photosynthetic CO_2 assimilation was confirmed in terms of increased carbohydrate production with the increase in Mg^{2+} dose (Figure 4).

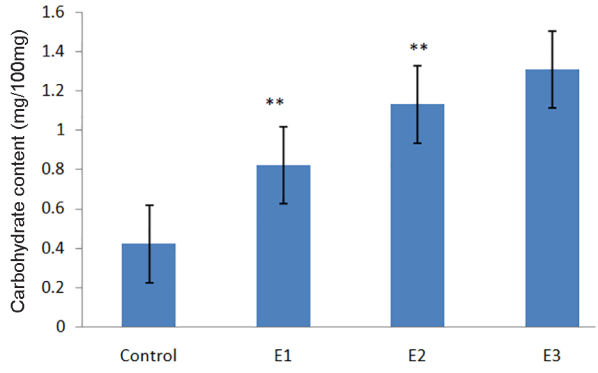


Figure 4. Change in Carbohydrate content in the experimental plants

Malondialdehyde (MDA) accumulation was reported in rice (Ding et al., 2008), as a general indicator of lipid peroxidation under low Mg^{2+} availability conditions. According to the study conducted by Hauer-Jákli and Tränkner (2019), the levels of ROS increased by 31% under Mg^{2+} deficiency. In the work by Kobayashi et al. (2018) there was an increased level of oxidative stress in Mg^{2+} deficient rice plants. MDA is an important by product of lipid-peroxidation during oxidative stress. Hence sufficient Mg^{2+} supply is required for plants to reduce oxidative stress. It is also been reported that As^{3+} causes oxidative stress in plants. In the present study, MDA content analysis also showed a similar trend (Figure 5). The control sample with only As^{3+} exposure had shown maximum stress in terms of highest MDA content. However,

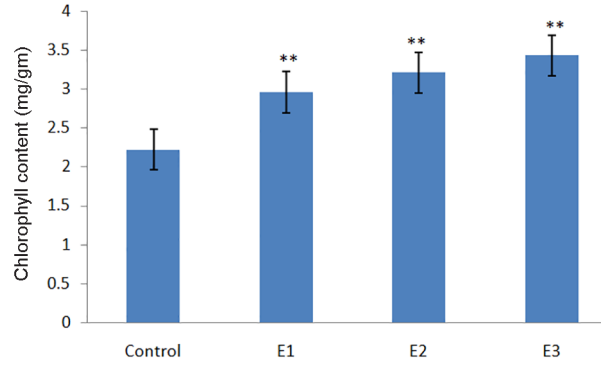


Figure 3. Change in Chlorophyll content in the experimental plants

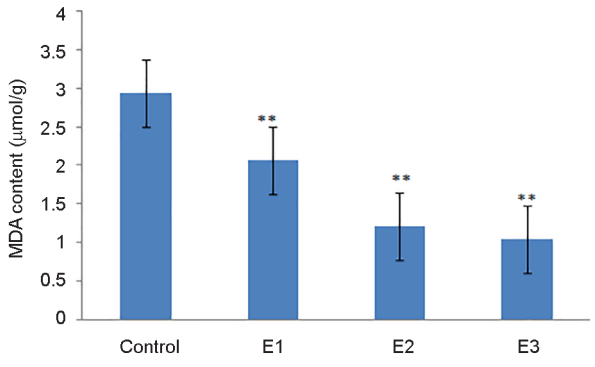


Figure 5. Change in MDA content among the experimental plants

with increasing Mg^{2+} fertilizer dose, MDA content had decreased considerably. Hence, it could be hypothesized that Mg^{2+} might have played some positive roles in rice plants to withstand the increment of MDA.

Studies on rice plant have shown that magnesium (Mg^{2+}) has a significant impact on the nutritional quality of rice. With sufficient magnesium (Mg^{2+}) supply in rice plant the total protein content significantly increases. It has also been observed in brown rice that total amino acids is increased and the contents of two kinds of limiting amino acids, lysine and threonine, of cereal protein is also increased leading to improvement of the nutritional quality of the produce (Ding, 2002). The experimental results (Figure 6) showed that the control plants with only As^{3+} treatment had minimum protein content but with increased Mg^{2+} content in other plants the protein content had increased which corroborate the findings by previous researchers.

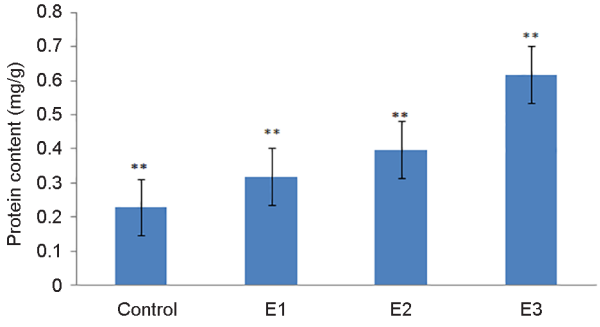


Figure 6. Comparison of protein content in different experimental plants

2.3. Molecular interaction study

The docking interaction studies between 6OCE protein with both As^{3+} and Mg^{2+} showed the value –1.37 and –1.31Kcal/mol respectively, it seems that the interaction with As^{3+} was slightly better. It was further observed that in the presence of Mg^{2+}, the uptake of As was increased as the value of binding interaction was enhanced from –1.37 to –1.39 Kcal/mol (Figure 7c).

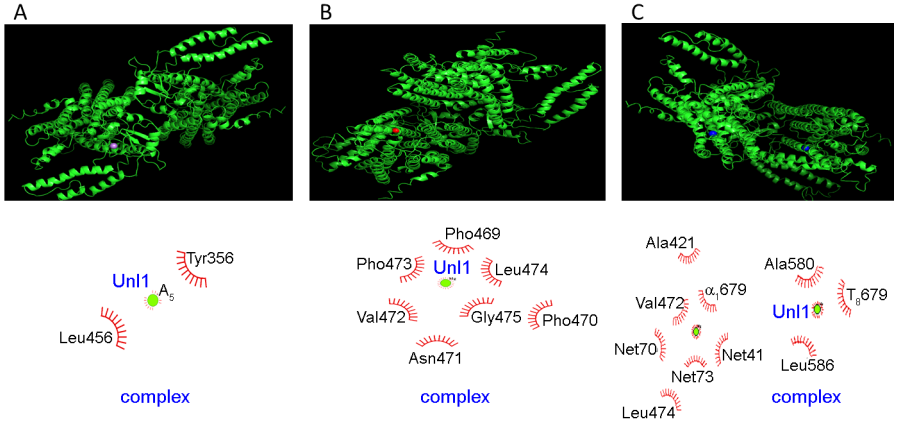
Table 2. Molecular interaction between transport protein with As^{3+} and Mg^{2+} ions

Interaction	Binding energy (Kcal/mol)	Amino acid
Arsenic vs 6OCE	–1.37	Tyr 356, Leu 456
Magnesium vs 6OCE	–1.31	Phe 469, Phe 473, Val 472, Asn 471, Gly 475, Leu 474
Magnesium vs 6OCE vs Arsenic	–1.39	**With Magnesium**: Val 472, Gly 475, Phe 469 **With Arsenic**: Tyr 356, Leu 456, Ala 570

The ligplot revealed the stable interactions of Mg^{2+} and As^{3+} with transport protein via hydrogen bond as represented in Figure 7 and Table 2. It is interesting to highlight that although As^{3+} ion had shown competitive advantage over Mg^{2+} in *O. sativa,* but without effecting the plant which is justified from the biochemical parameters and it is possible because of application of Mg^{2+} fertilizer (Thakur et al., 2020; Gransee & Führs, 2013) in the cultivation process.

2.4. Implication of the work

Arsenic is a toxic metalloid naturally present in the soil. Rising soil As concentration is a major concern around the globe due to the health risk to plants, animals and human beings. Rice is the staple food in eastern part of India and soil Arsenic is a major concern here. The waterlogged field condition in rice cultivation makes it more prone to As^{3+} uptake. Mg^{2+} is an important macro nutrient required for normal growth of the plant. In the work we have found that adding Mg^{2+} along with commercial fertilizer (in this case 'N-P-K') improves health of the plant as well as reduces As^{3+} induced stress. Hence it can be an eco-friendly and sustainable solution to the global agricultural problem.



Note: Text marked in blue represents reviewers (both reviewer 1 and reviewer 3) comments.

Figure 7. Molecular interaction of transport protein of *O. sativa* (6OCE) (A) As^{3+} (B) Mg^{2+} (C) Synergistic interaction of Mg^{2+} and As^{3+}

Conclusions

The purpose of this work was to understand how the application of Mg^{2+} could be effective in reducing the As^{3+} toxicity in plants. In the present study, the docking interaction between As^{3+} and Mg^{2+} with the transporter protein revealed that plants accumulated a lower amount of Mg^{2+} compared to As^{3+} (Figure 7c). From the analysis of all the biochemical parameters, it could be concluded that the application of Mg^{2+} fertilizer showed effectiveness in reducing As^{3+} induced toxicity. Interestingly though the docking interaction showed more uptake of As^{3+} compared to Mg^{2+} but the biochemical parameters showed less stress in plants. This may be due to added Mg^{2+} doses, which led us to forecast that Mg^{2+} can act as a toxicity reducer for As contaminated plants. Further work in this direction might be employed to ascertain the optimum application of Mg^{2+} fertilizer in order to represent the best performances.

Conflict of interest

The authors do not have any conflict of interest in publication of this work.

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