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Molybdenum and sulphur ion mitigate arsenate and arsenite translocation in rice crop

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Abstract

This paper report on a hydroponics experiment that was conducted to investigate the effect of Molybdenum (Mo) and Sulphur (S) on As-V and As-III accumulation within the seedling of the rice cultivar IET-4786 (Shatabdi). The seedling were subjected to four concentration of Mo and S, i.e. full Hoagland solution (CH), Hoagland solution with high Mo and S, Hoagland solution with low Mo and S and Hoagland solution with Mo and S deficient with As-V and As-III contamination along with CH as a control treatment. Analysis of Mo, S and As species of the leaves and root of seedling indicated that uptake of As-V and As-III was significantly affected by both Mo and S. Mo nutrition make some complex with As in solution and prevent As entry in to the plant system. S in the form of sulphate nutrition plays an important role in regulating arsenic translocation from roots to leaves and arsenic translocation from roots to shoots was enhanced by sulphur deprivation.

Keywords: molybdenum, sulphur, arsenate, arsenite

Introduction

Arsenic (As) is an element that is nonessential for and toxic to plants. Groundwater contamination with arsenic is reported from many regions of the world, the most severe problems occur in Bangladesh, West Bengal, China and Taiwan [1]. Physiological and electrophysiological studies have shown that arsenate and phosphate share the same transport pathway in higher plants [2], with the transporters having a higher affinity for phosphate than for arsenate [3, 4]. Meharg and Jardine [5] suggested that arsenite may be taken up by aquaporin channels in plant roots. Recently, evidence that some plant aquaporin channels can mediate arsenite influx has been obtained from three independent studies [6, 7, 8]. Heuwinkel *et al.* [9] also found that P deficiency enhanced markedly Mo uptake by tomato plants and implied that molybdate was taken up by a phosphate transporter. Sims *et al.*[10] reported that molybdate anion ($Mo0_4^{2-}$) also behaves more like the sulfate anion (SO_4^{2-}) than the phosphate anion (HPO_4^{2-}) in soil, Mo significantly reduced P concentration of berseem and attributed this to the fact that both phosphates and molybdates were absorbed in anionic forms and they might compete with each other for the absorption sites [11] and uptake of molybdate by plants is known to be decreased in the presence of large amounts of sulfate, probably because both anions use the same transport system [12]. One argument for SO_4^{2-} uptake systems in plants is the fact that, in contrast to inhibition of molybdate uptake by large amounts of sulfate, molybdate supply does not affect sulphate uptake [13]. In plants, the uptake of molybdate may occur through sulfate transport proteins [14] as both molybdate and sulfate have similar chemical properties. In plants, sulfur (S) starvation can enhance Mo accumulation [15] or alternatively repress Mo uptake when supplied at increasing concentrations [16]. Reactions of arsenic salts with molybdate produced heteropoly compounds of various compositions and structures. Arsenic (V) heteropoly anions (HPAs) are well known: $[AsMo_{12}O_{40}]^{3-}$, $[AsMo_9O_{31}(OH_2)_3]^{3-}$ and $[As_2Mo_8O_{62}]^{3-}$. In an excess of arsenate ions, the following HPAs can form: $[As_2Mo_6O_{26}]^{6-}$, $[As_4Mo_{12}O_{50}H_4]^{4-}$and $[H4As_4Mo_4O_{26}]^{4-}$. With organoelement ions R_2AsO_2 (R = CH_3, C_2H_5, or C_6H_5) and $RAsO_3$, the following HPAs were obtained: $[R_2AsMo_4O_{15}H]^{2-}$, $[(RAsO_3)_2Mo_6O_{18}]^{4-}$ and $[(CH_3AsO_3)Mo_6O_{18}(H_2O)_6]^{2-}$ respectively [17].

Sulphur deprivation in nutrient solution decreased the concentrations of non-protein thiols in rice roots exposed to either arsenite or arsenate. The low sulphate-pretreated plants had a higher arsenic transfer factor than the high sulphate-pretreated plants. The results suggest that rice sulphate nutrition plays an important role in regulating arsenic translocation from roots to shoots, possibly through the complexation of arsenite-phytochelatins [18]. Phytochelatins (PCs) are thiol (SH)-rich peptides, whose production is induced by heavy metals [19, 20, 21, 22]. This hypothesis was supported by evidence of the formation of As-SH complexes both in vivo and in vitro [18, 23]. The gene encoding PC synthase (the enzyme responsible for the production of PCs from glutathione [GSH]) has recently been identified [20, 24]. Sulphur help in binding of As-III to sulfhydryl groups in GSH and PC in the detoxification of the metalloid indicates a critical importance for sulfur metabolism in determining plant survival in As-contaminated soils [25]. We used As-V and As-III arsenic species in our study to see whether there is any species of arsenic mitigate by differential concentration of molybdenum and sulphur during rice growth under hydroponic system.

Material and Methods

Plant material and culture

All experiment was conducted in Directorate of Research, Bidhan Chandra Krishi Viswavidyalaya, Kalyani, West Bengal.

Seed of hybrid rice (*Oryza sativa* L.) cultivar, IET-4786 (Shatabdi), were surface sterilized in 0.1% $HgCl_2$ (w/v) solution for 3 min, thoroughly washed with deionised water and then germinated in sterilized sand. At 18 days after germination, uniform seedlings were selected. The sand adhering to the seedling root was washed with deionized water, and the seedling were transferred to $2^{1/2}$-L plastic pots containing 2-L Hoagland nutrient solution with different dose of molybdate (M_{OH}= molybdate with $1^{1/2}$ of Hoagland solution, M_{OL}= molybdate with 1/2 of Hoagland solution, M_{O}-= molybdate deficient, CH= Complete Hoagland solution) and sulphur (S_H= Sulphur with $1^{1/2}$ of Hoagland solution, S_L= Sulphur with 1/2 of Hoagland solution, S- = Sulphur deficient). The composition of the complete Arnon and Hoagland [26] nutrient solution (CH) was 1.02 g/L KNO_3, 0.492 g/L $Ca(NO_3)_2$, 0.492 g/L $MgSO_4$,$7H_2O$, 0.23 g/L $NH_4H_2PO_4$ and micronutrients 2.86 mg/L H_3BO_3, 1.81 mg/L $MnCl_2$,$4H_2O$, 0.08 mg/L $CuSO_4.5H_2O$, 0.22 mg/L $ZnSO_4$,$7H_2O$, 0.09 mg/L H_2MoO_4,H_2O, 0.60 mg/L $FeSO_4$/tartaric acid.

The high and low Mo treatments were developed by adding molybdic acid 1.5 times and 0.5 times of their concentration in basic culture solution. In their deficient version the respective chemical was complete absent. Normal concentration of each of these elements meant its concentration as per complete Arnon and Hoagland.

The composition of high S (S_H) solution was 1.02 g/L KNO_3, 0.492 g/L $Ca(NO_3)_2$, 0.73 g/L $MgSO_4.7H_2O$, 0.23 g/L $NH_4H_2PO_4$ and other micronutrient concentration is same as CH. The composition of low S (S_L) solution was 1.02 g/L KNO_3, 0.492 g/L $Ca(NO_3)_2$, 0.243 g/L $MgSO_4.7H_2O$, 0.23 g/L $NH_4H_2PO_4$, 0.041 g/L MgO and other micronutrient concentration is same as CH. The composition of S absent (S) solution was1.02 g/L KNO_3, 0.492 g/L $Ca(NO_3)_2$, 0.23 g/L $NH_4H_2PO_4$, 0.079 g/L MgO and other micronutrient concentration is same as CH.

The rice seedling grew normally in this nutrient solution. The pH of the Hoagland nutrient solution was adjusted to 5.5 with 0.1 M NaOH or HCl. The seedlings were inserted in to the hole of a Styrofoam plate that was floated on the nutrient solution. The plants were grown in a net house. Two species of inorganic arsenic – As(III) in the form of Sodium arsenate ($Na_2HAsO_4.7H_2O$, M. W. = 321.01) and As(V) in the form of Sodium arsenite ($NaAsO_2$, M. W. = 129.91)- were added to the nutrient solution at 5 ppm concentration. After 7 days of treatment, the rice seedlings were analyzed for molybdenum, sulphur and total Arsenic content in both arsenate and arsenite treatment.

Further, in the order to analyse the mechanism underlying the toxic effect of the two species of arsenic on the absorption and utilization of nutrients in the rice seedling, the treatment dose of two arsenic species was fixed at 5 ppm. The rice seedlings were cultured as same as described above. After 7 days of treatment, leaves and root from the rice seedling sample was mixed with triacid mixture (HNO_3:H_2SO_4: $HClO_4$ with 10:4:1 ratio) in an Erlenmeyer flask for sulphur treatment sample and diacid mixture (HNO_3: $HClO_4$ with 9:4 ratio) for sulphur treatment sample. After an overnight reaction, the content of the flask were gently boiled on an electric heater for digestion. The entire digestion process lasted 3-4 hr after complete digestion, the solution was diluted with double distilled water and transferred in to acid-washed plastic bottle; this solution was used for analyzing the molybdenum, sulphur and arsenic content of the sample. Each treatment was performed in triplicate.

Analysis of the total arsenic in plant sample

Take 2 ml aliquot in a plastic test tube and added 10% HCl of total volume. Add 1 ml KI and 1 ml Vit.-C than volume make up 10 ml. Total arsenic of the digested rice seedling (root and leaves) samples were analyzed by flow injection hydride generation atomic absorption spectrophotometer (FI-HG-AAS, Perkin Elmer Aanalyst 400) using external calibration through arsenic as standard. The optimum HCl concentration was 10% v/v and 0.4% NaBH4 produced the maximum sensitivity. For each sample three replicates were taken and the mean values were obtained on the basis of calculation of those three replicates.

Analysis of the total molybdanum in plant sample

Take 10 ml aliquot sample in to a 25 ml volumetric flask and add 5 ml HCl. Add 1.5 ml of 10% potassium thiocyanate solution and 8 ml of acetone. Make up the volume to 25ml with water and mix. Cool to room temperature and mix. If turbidity is present, centrifuge or filter through whatman No. 42 filter paper of a portion of the solution and read at 470 nm against a reagent blank. Draw the standard curve using 0.1-2 ppm of sulphur (as $(NH_4)_6M_{O7}O_{24}.4H_2O$) under identical condition [27].

Analysis of the total sulphur in plant sample

Take 10 ml of the aliquot sample, add 1 ml of stabilizing reagent ((95%etanol: glycerol): mix ethanol and glycerol in 8:2 ratio (v/v)) and 0.5 g of $BaCl_2$ mix it and make up the volume to 25 ml with water. Measure the absorbance at 430 nm against the blank prepared similarly without the sample. Draw the standard curve using 0.1-2 ppm of sulphur (as K_2SO_4) under identical condition [28].

Experiment design

A hydroponic experiment was conducted at Directorate of Research, Bidhan Chandra Krishi Viswavidyalaya, Kalyani, West Bengal. The experiment was laid out in CRD design with treatment combinations in three replications.

Calculation of factor

Total arsenic or nutrient content = Shoot content + Root content

Total arsenic uptake (mg) = (Total arsenic content of treatment combination with arsenate or arsenite (mg) – (Total arsenic content of normal treatment without arsenate or arsenite (mg)

Arsenic translocation (mg) = Shoot arsenic content with arsenate or arsenite (mg) – Shoot arsenic content without arsenate or arsenite (mg)

$$\text{Arsenic translocation factor (\%)} = \frac{\text{Arsenic translocation (mg)}}{\text{Total arsenic uptake (mg)}} \times 100$$

Statistical analysis

Experimental data were analyzed statistically by using the windows-based SPSS 12.0 package at 95% significance level. The experimental data for the characters were subjected to the variance analysis appropriate to a CRD design.

Result and Discussion

Result

Analysis of the Mo, S and as content of rice seedlings leaves and root after treatment with different combination of Mo and S with arsenate and arsenite. The results show that arsenate and arsenite of leaves and root of rice seedling interact significantly with various concentrations of Mo and S.

The variations in roots and leaves as content due to Mo and arsenic treatment combinations were statistically significant indicating substantial effect of Mo concentration and as species in nutrient solution on as content in leaves and roots are presented in Table 1. Roots As content was highest under T_4 (22.30) followed by T_8 (22.18) and lowest under T_7 (18.26) as treatment. Leaves As content was highest under T_4 (4.9) followed by T_6 (4.90) and lowest under T_1 (3.80) as treatment. Total As content was highest under T_4 (27.26) followed by T_8 (27.06) and lowest under T_7 (22.11) as treatment. The values of arsenic uptake under arsenite contaminated condition were 26.46, 26.65, 26.85 and 25.55 respectively under high, normal, low and zero level of Molybdenum which were higher than the corresponding values (21.74, 21.70, 22.08 and 21.73) under arsenate contaminated condition. The values of arsenic translocation under arsenite contaminated condition were 4.68, 4.74, 4.80 and 4.75 respectively under high, normal, low and zero level of Molybdenum which were higher than the corresponding values (3.65, 3.70, 3.72 and 3.73) under arsenate contaminated condition. The values of translocation factor under arsenite contaminated condition were 17.69, 17.79, 17.88 and 18.59 respectively under high, normal, low and zero level of Molybdenum which were higher than the corresponding values (16.79, 17.05, 16.85 and 17.17) under arsenate contaminated condition (Figure 1).

The variations in roots and leaves as content due to S and arsenic treatment combinations were statistically significant indicating substantial effect of S concentration and as species in nutrient solution on as content in roots and leaves are presented in Table 2. Roots As content was highest under T_2 (22.53) followed by T_8 (22.39) and lowest under T_5 (17.15) as treatment. Leaves As content was highest under T_4 (4.97) followed by T_2 (4.91) and lowest under T_1 (3.57) as treatment (Table 2). The values of arsenic uptake under arsenite contaminated condition were 27.00, 26.80, 26.05 and 25.19 respectively under high, normal, low and zero level of sulphur which were higher than the corresponding values (21.71, 21.65, 21.54 and 20.96) under arsenate contaminated condition. The values of arsenic translocation under arsenite contaminated condition were 4.76, 4.71, 4.82 and 4.66 respectively under high, normal, low and zero level of sulphur which were higher than the corresponding values (3.42, 3.74, 3.95 and 4.10) under arsenate contaminated condition. The values of translocation factor under arsenite contaminated condition were 17.63, 17.57, 18.50 and 18.50 respectively under high, normal, low and zero level of sulphur which were higher than the corresponding values (15.75, 17.27, 18.34 and 19.56) under arsenate contaminated condition (Figure 2).

Table 1: Arsenic content (µg) in rice seedling roots and leaves under different molybdenum treatments

Sl. No.	Treatment	Root arsenic content (µg)	Leaves arsenic content (µg)	Total arsenic content (µg)
T_1	M_OH + As-V	18.35	3.80	22.15
T_2	M_OH + As -III	22.04	4.83	26.87
T_3	M_OL + As -V	18.62	3.87	22.49
T_4	M_OL + As –III	22.30	4.95	27.26
T_5	M_O- + As-V	18.26	3.88	22.14
T_6	M_O- + As-III	21.06	4.90	25.96
T_7	M_{ON} + As-V	18.26	3.85	22.11
T_8	M_{ON} + As-III	22.18	4.89	27.06
T_9	M_{ON} (CH)	0.26	0.15	0.41
Mean		17.93	3.90	21.83
SEm (±)		0.02	0.02	0.03
CD (0.05)		0.06	0.07	0.09

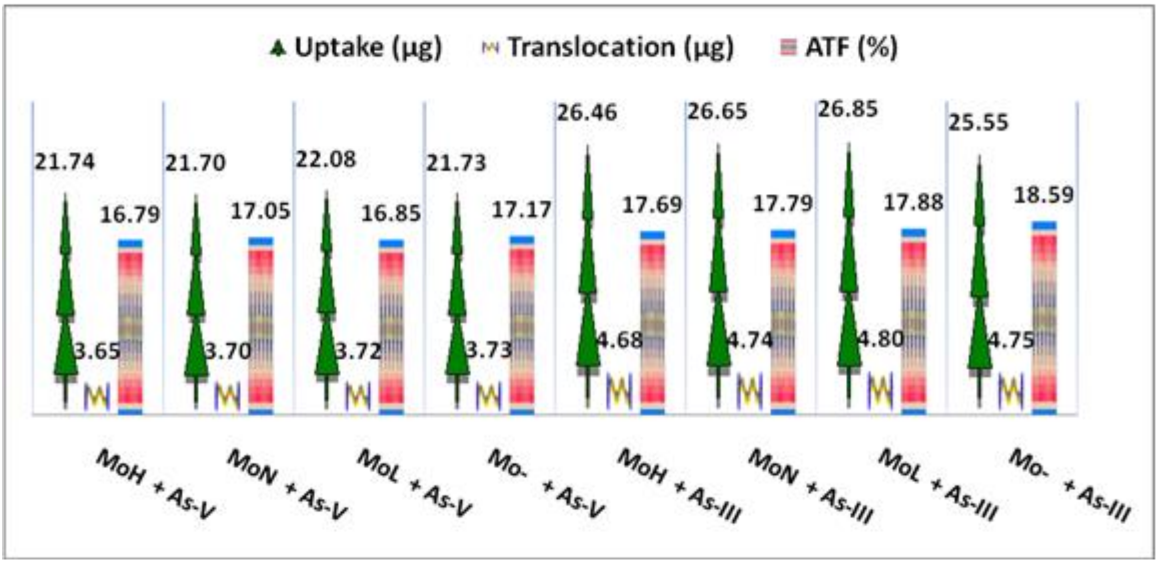

Fig. 1: Arsenic uptake (µg), Arsenic translocation (µg) and Arsenic translocation factor (%) by rice seedlings under different concentration of molybdenum nutrient solution

Table 2: Arsenic content (µg) in rice seedling roots and leaves under different sulphur treatments

Sl. No.	Treatment	Root arsenic content (µg)	Leaves arsenic content (µg)	Total arsenic content (µg)
T_1	S_H + As-V	18.58	3.57	22.15
T_2	S_H + As –III	22.53	4.91	27.44
T_3	S_L + As –V	17.89	4.10	21.98
T_4	S_L + As –III	21.52	4.97	26.49
T_5	S- + As-V	17.15	4.25	21.40
T_6	S- + As-III	20.82	4.81	25.63
T_7	S_N + As-V	18.19	3.89	22.09
T_8	S_N + As-III	22.39	4.86	27.24
T_9	S_N (CH)	0.30	0.15	0.44
Mean		17.71	3.95	21.65
SEm(±)		0.03	0.01	0.04
CD(0.05)		0.08	0.04	0.11

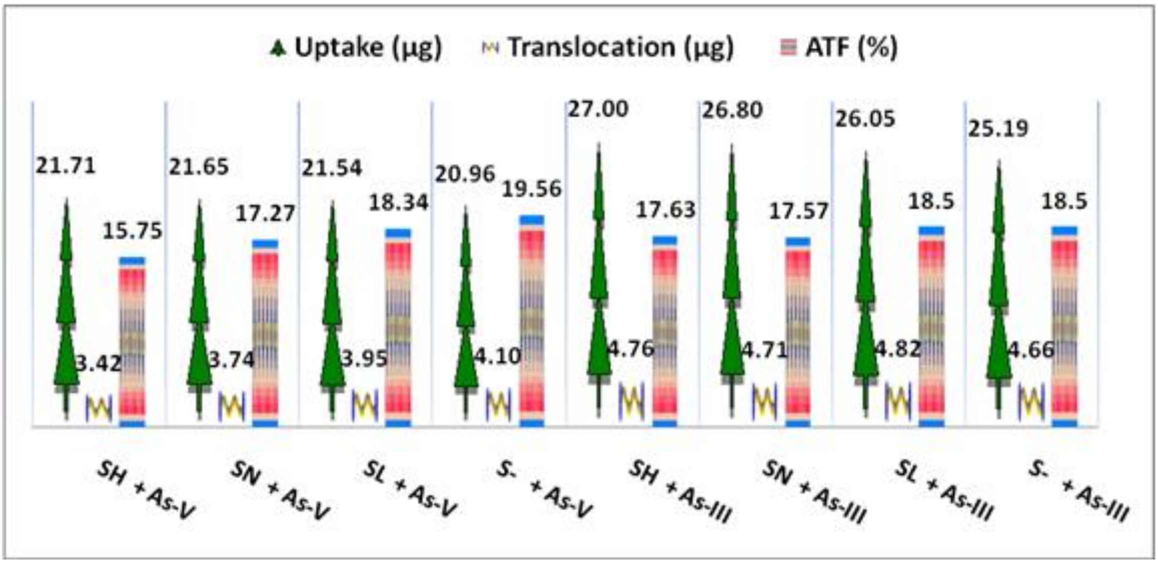

Fig. 2: Arsenic uptake (µg), Arsenic translocation (µg) and Arsenic translocation factor (%) by rice seedlings under different concentration of sulphur nutrient solution

Discussion

Discussion end point such as molybdenum (Mo), Sulphur (S) and Arsenic (As) uptake used for investigating uptake, translocation and mitigation response of as in the studies on the response of plant to Mo and S. Reduction in the As-V and As-III are typical response of plant to Mo and S. In our study, As-V and As-III were found to be significantly affected by the Mo and S used. The significant reduction in As-V and As-III with increasing concentration of Mo is attributed to the fact that the arsenic was may be the point of contact with Mo in the nutrient medium. Koshcheevaa *et al.* [17] also reported that arsenic salt make some heteropoly compound with Mo under hydroponic condition and inhibit transport of arsenic within plant system. The significant reduction in leaves As-V and As-III with increasing concentration of S attributed to the fact that sulphate nutrition plays an important role in regulating arsenic translocation from roots to shoots, possibly through the complexation of arsenic-phytochelatins [18].Most research results indicated that As-III exerted more toxic effects on the growth of rice plants than As-V [29].

The results obtained in this paper are well consistent with other studies, but the mechanism was unclear yet. In this study, the change of arsenate and arsenite content and uptake of Mo and S involved in the mitigation of As-V and As-III in rice seedling after treatment of different concentration of Mo and S were investigated. The results revealed that the uptake of As-V and As-III in rice seedling varies with different concentration of Mo. (Figure 1). Mo decreased the contents of As-V and As-III both in roots and leaf of rice seedlings. Mokgalaka-Matlala *et al*. [30] also reported that Mo was reduced in As-treated plants. As-III uptake and translocation affected by different concentration of Mo more than As-V did and translocation of As-V and As-III during exposure significantly reduced with increase Mo concentration that reflects the As translocation from root to leaves differed with Mo concentration (Figure 1). Arsenic translocation factor (%) constantly reduced with increase in Mo concentration in As-III contaminated condition. In case of As-V, Arsenic translocation factor (ATF) varies with Mo concentration but higher Mo having lower ATF. It indicates that higher Mo reduced ATF (Figure 1). Result of as content, uptake, translocation, ATF vary significantly with Mo concentration indicating that substantial impact of Mo concentration.

In case of S treatment, translocation of As-V and As-III in rice seedling from root to leaves decrease with increase in concentration of S (Figure 2). Zhang *et al.* [18] also found that rice sulphate nutrition plays an important role in regulating arsenic translocation from root to shoots, possibly through the complexation of arsenite-phytochelatins. Root As content continuously increases with increase in S content under As-V and As-III contaminated condition. Zhang *et al*. [18] also reported that the high sulphate treatments had higher root as concentrations than the low sulphate treatments, either expose to arsenite or arsenate. Leaves As content continuously decrease with increase in S content under As-V contaminated condition but in case of As-III, As content increase with low sulphur (S_L) but decrease with high sulphur (S_N and S_H). As-III uptake affected by different concentration of S more than As-V did (Figure 2) and translocation of As-V during exposure significantly reduced with increase S concentration that reflects the As translocation from root to leaves differed with S concentration (Figure 2). Under As-III contaminated condition, as translocation increase with low sulphur (S_L) but decrease with high sulphur (S_N and S_H). Arsenic translocation factor (%) constantly reduced with increase in S concentration in As-V contaminated condition. In case of As-III, ATF reduced with increase in S concentration but high S concentration (S_H) increase ATF as compared normal S (S_N) (Figure 2). Duan *et al.* [23] also reported that the higher transfer factor of total arsenic in the plants with low sulphate treatments indicates that arsenic translocation from roots to shoots was enhanced by sulphur deprivation; this was the case in both arsenite and arsenate exposure.

Our results also showed that in rice seedlings, Mo and S principally affected the uptake and translocation of As-V, whereas As-III also principally affected by Mo and S but As-V shows highly significant response compared to As-III. It hints that Mo and S could influence the accumulation of different As-V and As-III in rice seedlings and decrease the poison of As. So reduction of as toxicity on rice by nutrient regulation will be further studied.

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