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SUMMARY OF DOCTORAL THESIS

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Title: Studies on the Increase in Arsenic Concentration in Brown Rice Due to High Temperatures During the Ripening Period and Countermeasures for Reducing the Arsenic Concentration by Applying Soil Modifiers
(登熟期の高温による玄米ヒ素濃度の上昇ならびに種々の土壌改良資材施用によるその低減対策に関する研究)

Arsenic (As) is a non-essential, potentially ubiquitous metal, naturally occurring in the environment with a relatively low concentration. Rice, the primary dietary source of inorganic As, is more concentrated As in grain than other cereals, despite its high essential nutritional content like carbohydrates, protein, vitamins, and fiber. Increased air temperature would amplify the risk of increasing toxic inorganic As (iAs) accumulation in rice grain using multiyear statistical analysis. Considering the As toxic effect, many authentic mitigation techniques and tests have been followed to reduce As concentration in rice. However, the effect of high temperature on As accumulation in rice grain during the ripening period is not research experimentally. This thesis aimed to clarify the effect of high temperature during the ripening period on the elution of As in soil solution from soil solid phase and on the concentration of As species in brown rice using temperature-gradient chamber (TGC). Furthermore, it was verified whether the application of soil conditioner (silicate and iron) was effective in reducing the arsenic concentration in brown rice even at high temperatures.

This thesis gave some critical insights for conducting three experiments. The growing conditions in these experiments were common, and the cultivation and high-temperature treatment of rice were carried out as follows. Before transplanting Wagner pots were filled with 3-kg As-uncontaminated gray lowland paddy field soil and 2.8 g of a basal compound fertilizer used in every pot, which each pot contained N 14%, P_2O_5 14%, and K_2O 14%. Calcium silicate and converted furnace slag (CFS) were used as Si treatment and Fe treatment, respectively. Rice seedlings that were approx. 25 days old were transplanted to the Wagner pots and kept those with flooding conditions until harvest. One week after heading, all rice plants were moved into TGC for temperature-treatment, where the temperature treatment section separated, followed by ambient, mildly-high, moderately-high, and super-high temperature treatment to detect the temperature variation.

Air and soil temperature difference was observed in the TGC in the range of 2-3.2 °C compared to ambient temperature. The high temperature significantly increased the total As concentration in the soil solution during the ripening period. The concentrations of As in the soil solution during the middle ripening period (14-28 days after heading) were tended to be higher than those of the early ripening period (7 days after heading) and harvesting period (41 days after heading).

There was a significant negative correlation between the brown rice yield and the air and soil temperatures, and the increase in air and soil temperatures resulted in a decrease in the yield. The reduction in yield was significantly mitigated by applying calcium silicate and CFS. The concentration of As in the brown rice was significantly positively correlated with the air and soil temperature, and the concentration of As increased with increasing air and soil temperatures. When calcium silicate and CFS were applied, the concentration of As in brown rice was significantly lower at all temperature ranges, and those applications were effective in reducing the arsenic concentration even at high temperatures. These results suggest that the application of soil modifiers including silicate and iron may help mitigate the decrease in yield and the increasing As concentration in brown rice even under high-temperature conditions.

The total As concentration in each part of the straw, especially in node 1, node 2, and internode 2, was found a significant regression linearity correlation with the temperature when the average day time and maximum temperature were high. Although no significant linear relationship has been found between temperature and carbon concentration at any parts of the rice plant, indicating that there is no unique accumulation mechanism in those parts of rice plants for carbon translocation.

The accumulation of As and carbon significantly reduced with increasing temperature in the brown rice due to the ripening period shorten when the temperature increased highly. The distribution ratio of As and carbon had been found to decrease significantly in brown rice when the temperature increased. A negative correlation has been found between the concentration of As and carbon distribution ratio in brown rice. A significant positive correlation was found between the As/carbon ratio of the distribution ratio and the concentration of As in brown rice, indicating the decrease in the carbon translocation rate from straw to the grain may increase As concentration in brown rice. In other words, the main factor of the increase of As concentration in brown rice under high-temperature conditions is considered to be insufficient dilution by carbon and damaged node function which trap As. Our findings highlighted that elevated temperatures might increase the risk of dietary As exposure in rice by decreasing the dilution effect by carbon and decrease of the node function to accumulate more As.

From this study's results, the yield decreased with increasing temperature in the ripening period due to the suppression of carbohydrate translocation during this period. The amount of carbon accumulation and the carbon distribution ratio were lower in the high-temperature treatments. Similarly, because As is transferred to brown rice through the sieve tube, the amount of As accumulated in brown rice and the distribution rate of As were examined, compared to carbon. A significant negative correlation was found between the total As concentration in each part of the straw, especially in node 1, node 2, internode 2, and the average daytime temperature and maximum during the ripening period. These results indicate that the ability to prevent As translocation to brown rice, present in the nodes, may be reduced by high temperatures. No significant linear relationship has been found between temperature and carbon concentration in any parts of the rice plant, indicating no unique accumulation mechanism in those parts of rice plants for carbon translocation.

The accumulation of As and carbon significantly reduced with increasing temperature in the brown rice, as the ripening period shortened when the temperature increased. The effect of high temperature on As distribution ratio in straw was not as clear as that of carbon. However, it was observed that the distribution ratio of As to brown rice decreased with increasing temperature, which was also the case with carbon and suggests that the effect of high temperature on the transfer and accumulation of carbon and As in brown rice may be different. The distribution ratio of carbon tended to increase with a high temperature in all parts of the straw. In contrast, As showed a similar trend to carbon in the straw under internode 3, but there was no clear relationship with high temperatures in other parts. The negative correlation between the decrease in carbon distribution ratio and As concentration in brown rice was significant. This suggests that carbon translocation flow significantly affects As concentration in brown rice; As concentration decreases with high carbon translocation flow and increases with low carbon translocation flow. The As concentration increases at higher temperatures, suggesting that the translocation inhibition ratio due to high temperatures differs between As and carbon because the amount of As transferred to brown rice also decreases at higher temperatures. In other words, higher temperature causes less As dilution by carbon and higher As concentration in brown rice.

The significant difference between carbon and As translocation is that there is no difference in carbon concentration and no exclusion or accumulation mechanism in the translocation. If this sequestration mechanism is affected by high temperatures, the As transfer into brown rice could be accelerated. On the other hand, the final amount of As accumulation was smaller at higher temperatures than under ambient conditions. This may be due to the shortened ripening period caused by high temperatures. Therefore, it would be necessary to clarify the As translocation rate by considering the As exclusion mechanism relative to temperature over the ripening period. Furthermore, breeding rice varieties that are resistant to high-temperature ripening effectively reduced As concentration in rice because carbon's dilution effect may reduce As concentration.