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）

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Dedication

I dedicate this thesis to my husband and my children for their love and support through all whose owes can never be fulfilled. I also dedicate it to my parents and my siblings who have always supported me with their prayers, love, and encouragement.

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List of Abbreviations

As (III) Arsenite

As (V) Arsenate

DMPS 2-3-dimercapto-1-propanesulfonate

ICP-MS Inductively Coupled Plasma Mass Spectrometry

HPLC/ICP-MS High Performance Liquid Chromatography

HCL Hydrochloride Acid

HNO_3 Nitric Acid

MAFF Ministry of Agriculture, Forestry and Fisheries

CRM Certified by the Reference Material

HTT High Temperature Treatment

Chapter 1

Introduction

This chapter provides an overview of the arsenic contamination in rice grain. Recently, studies also focused that due to the high temperature, iAs increases in rice grain and the importance of rice in the agriculture of world. This chapter also presents an estimate of the mass of arsenic abstracted with high temperature effect to decrease the rice grain yield and risk for rice market.

1. General

Arsenic, regarded as a colorless, heavy toxic, and odorless mobile element metals and founds everywhere at trace levels in nature (Lombi et al., 2002). It is naturally occurring as a metalloid in the form of inorganic or chemical compounds (Mólgora et al., 2013; Harisha et al., 2009). Although, it exists in two different chemical types according to the base of physical, chemical, and toxicology properties: organic and inorganic. When arsenic combines with chemical elements, including oxygen, chlorine, and sulfur, it’s called inorganic arsenic (iAs). On other hand, when a combination of arsenic with carbon and hydrogen, in that time it's called organic arsenic. There are some many arsenic species are found in air, soil, and water (Figure 1.1). Besides, it arsenic changes structure, behavior, and chemical bonds with various species by attaching or detaching in the air, water, or soil. Even, by binding to micro pieces in the air, it can move freely for many days in nature (Mandal et al., 2002). More than 99 % of the As in the world is found in rocks, mainly in silicate minerals, where As substitutes Al, Fe, and Si (Bhumbla and Keefer, 1994), and sulfur commonly associates with As (Duker et al., 2005). Olson et al. (2014) reported that arsenite (trivalent) and arsenate (pentavalent) are the major types of inorganic arsenic that existing in the environment (water, soil, and foods). According to the chemical analysis of arsenic forms evidences that comparison on inorganic arsenic compounds are generally toxic and harmful than organic arsenic forms in which chemical compounds are not quickly eliminated in the human body. Some of the arsenic forms such as MMA and DMA are recognized as organic arsenic, these can be reformed in iAs. In some cases, DMA has the presence as iAs forms in human urine at the high levels, while DMA also exits in food as organic forms (Horner and Beauchemin, 2013; Wei et al., 2014). Its presence as exceeding the normal level in food composites could be a potential risk to the health of both humans and animals (Al Rmalli et al., 2005, Rintala et al., 2014, Zhao et al., 2010a)

Arsenic (As) is a heavy toxic metalloid, remarked as ubiquitous in the environment and considerable global groundwater contaminate in the environment. Now it is presenting as an international issue (Brammer et al., 2008). Arsenic is found not only in the ground and contaminated drinking water but also found in rice, meat, fruits, vegetables, etc that may lead to human arsenic toxicity by excessive exposure to arsenic. Agricultural crops which human takes in their daily food are damaged when a higher concentration of arsenic in irrigation water is supplied from the soil and accumulated by plants from root to grain where arsenic is available (Abedin et al., 2002).

Based on its chemical compound, released into the environment in natural processes (volcanoes emissions, withering rocks, and discharge from hot springs) and anthropogenic activities (mining, metal processing smelting, burning of coal, industrial applications, and use in insecticides, pesticides, and herbicides (Table 1.1). It is mainly severe occurring in certain rivers and deltas in east and south Asia and South American countries (Kobya et al., 2020). Especially, As contamination mass poisoning in Bengal delta regions (Meharg, 2005), recognized as the largest As contamination flat in history reported by WHO (2010). There are many countries with severe occurring As contamination such as the natural arsenic sources of the specific regions in Bangladesh, India, Thailand, and the United States (Figure 1.2). Arsenic contamination of regions to regions can be varied because of the As level adopted into the soil. Due to its cumulative nature, As tends to be most toxicity in human health, a leading cause of serious suffering from fatal and carcinogen diseases as well as adverse effects on cardiovascular, neurological, hematological, renal, and respiratory systems via taking their daily foods and drinking water (Figure 1.3) (Martinez et al., 2011; Sahoo et al., 2013; Gupta et al., 2017). The impacts of As poisoning is becoming wide-ranging that affect human health because of its source, route, ranging level in the world. Thus, the pathway of As from soil to water or rice may apply to human health risk to people living in As contaminated areas.

Rice is the most severely affected staple food crop to arsenic contamination as compared with other crops like wheat, barley, maize, and barely due to its cultivation in flooded conditions (Williams et al., 2007). Nevertheless, not only growing conditions but also the biological of rice make as the highest accumulated crop of As (Zhao et al., 2013). Figure 1.4 showed arsenic mechanisms in the paddy field. Arsenic can seriously affect the growth of rice plants after rooting translocation, as symptoms of stunted growth, brown spots, and leaf snoring, and arsenic toxicity containing > 60 mg^{-1} total soil arsenic (Bakhat et al., 2017). A rise in arsenic levels of soil from 12 to 60 mg kg^{-1} in traditional Bangladeshi paddy fields resulted in lower rice yields of 7.5 to 2.5 t ha^{-1} in Zavala and Duxburey (2008), and Panumullah et al. (2009). Even, As concentration increasing at a higher level, arsenic interrupts natural metabolism, and transpiration intensity is reduced when As exposure in rice. Thus, As adversely affects the rice plant metabolism to stop the growing system, and finally, rice plants become death. Wang et al. (2015) found that As accumulated up to 2 mg kg^{-1} in grain and 92 mg kg^{-1} in the straw when rice grown in As contaminated areas that are more excessive level compare to tolerable limits. Abedin et al. (2002) suggested that the higher concentration of As (8 mg of As L^{-1}) treatment significantly decreased plant height, grain yield, the number of filled grains, grain weight, and root biomass, while the arsenic concentrations in the root, straw, and rice husk increased significantly compared to another low As treatments. Azad et al. (2012) stated that tillers number, panicles number, panicle length, and grain yield were significantly decreased by 4 mg of As L^{-1}. Thus, Arsenic (As) is treated a non-essential, toxic metalloid that is concentrated in rice grains and arsenic accumulation in rice grains becoming is a serious issue both for reducing rice yield and quality. Now we details discussed As mechanisms in rice plants.

2. Arsenic uptake by rice plants

Rice is a dominant contributor to inorganic arsenic in humans daily intake their diet (Meharg et al., 2006) where it has been taken staple food especially in Asia, consumed and production approximately 90% (Mousavian et al., 2012). Therefore, the regions of rice resourcefulness and diversity may face highly contamination of arsenic and serve as a vital source of As exposure in humans (Chen et al., 2017). Arsenic concentration is presented as higher compared to other cereals crops such as wheat and barley (Williams et al., 2007; Hajsak et al., 2015). Due to the natural cumulative, rice plants are accumulated excessive arsenic, regarded as another important source of arsenic exposure (Melkonian et al., 2013). In addition, high levels of arsenic in water and soils lead to the elevation of arsenic bioavailability in rice plants (Abedin et al., 2002). Thus, the amendment of the available higher level of arsenic in soil that has been disrupted normal growth in rice. Some studies were followed in their results arsenic concentration intake in grain was rice>wheat>barely (Su et al., 2010). Because rice is mainly cultivated in flooding conditions. It is important for clarifying the arsenic levels in soil and water, soil conditions, and the mechanisms of arsenic uptake by rice plants because these conditions are associated with arsenic levels in rice grain. Figure 1.5 shows As availability on root surface.

2.1. Uptake of inorganic arsenic species

There are two mechanisms follow the uptake of iAs by rice roots. Among different forms of As in soil, As (V) is the predominant phyto available form in aerobic soils: from soil solution to aerial parts of the rice accumulated through the high affinity of phosphate transporter system (Wu et al., 2011) and loaded into xylem form vessels by phosphate transporter (PHT) proteins (Zhao et al., 2010b; Wu et al., 2011). Rice has 13 OsPT genes (*OsPT1* to *OsPT13*), the physiological roles of each OsPT in As transporter and all of those genes have high affinity to rice roots that were recognized as phosphate transporter gene family (Paszkowski, 2002). Among of those genes, *OsPT1* and *OsPT8* have a high affinity in arsenic transport in different parts of rice (Kamiya et al., 2013; Wang et al., 2016). OsPT1 has been highly expressed both in shoot and roots, and its expression was not affected by phosphate deficiency. Even, *OsPT1* showed higher constitutively in rice plants and expression suppressed by As (V). Thus, *OsPT1* is contributed to As (V) accumulation in higher than other *OsPTs* (Kamiya et al., 2013). Wang et al. (2016) found that OsPT8 plays an important role in As (V) uptake as protein transporter into rice roots and a profound toxic effect on root elongation was exerted after arsenate uptake mediated by OsPT8. Moreover, overexpression of *OsPT8* resulted in an enhanced arsenic accumulation in rice plants (Wang et al., 2016). Begum et al. (2016) suggested that a consistent decrease in tissue P concentration and expression of phosphate transporters (*OsPT8, OsPT4, OsPHO1:2*) under both high and low P supply in rice due to As stress. Besides, a simultaneous increase in phytochelatin concentration in rice roots was also observed under As exposure, indicating that phosphate transporter, and enhances phytochelatin-mediated As sequestration to vacuoles in root cells, limiting As translocation to shoots. Figure 1.6 shown As and species translocation mechanism from root to shoot grain.

The secondary route is arsenite taken up by aquaporin channels in root cells (Ma et al 2008). Arsenite enters through Lsi1, a nodulin 26 like intrinsic protein (*OsNIP2;1*), a major influx transporter for silicic acid (Li et al. 2009a; Ma et al., 2006), while another protein Lsi2, a silicon efflux transporter mediate arsenite efflux to the xylem in rice (Gonzalez et al., 2014). Lsi2, a significantly decreased rate of arsenite transport to xylem and accumulation in shoots and grain were found (Ma et al., 2008). Arsenite is taken up by the root cells; some of its instantly released into the rhizosphere by the bidirectional function of the Lsi1 protein channels (Zhao et al., 2010b). Inside plant tissues, arsenite is reduced to arsenite; arsenite is sequestered into root vacuoles or is translocated to the shoots and it is disseminated to various organs (Zhao et al., 2010b). The transport of that complex across the tonoplast is believed to be mediated by a C-type ATP binding cassette transporter (Song et al., 2014), which may therefore be of paramount importance for As resistance in plants.

2.2. Uptake of organic arsenic species

Considerable quantities of methylated arsenic species DMA and smaller amounts of MMA are found due to the microbial transformation of inorganic species to organic forms (Meharg et al., 2009). Therefore, DMA and MMA, are taken up at a much slower rate by the root than iAs, due to the lower affinity of transporter for organic As (Abedin et al., 2002; Raab et al., 2007). In furthermore, arsenic methylated species (MMA, DMA, etc.) are present from soil to plants when microbial bacteria methalysed iAs species in anaerobic conditions. MMA uptake is also partially mediated by the silicic acid transporter Lsi1, while the specific transport pathways of DMA are not yet clear (Li et al., 2009b; Carey et al., 2011). Plants such as rice appear to lack the ability to methylate As, but instead take up methylated As from the soil (Jia et al., 2013). Inorganic arsenic species are efficiently taken up by roots than methylated arsenic species, although the translocation rate in rice shoots of inorganic arsenic species is much lower than methylated arsenic species (Raab et al., 2007). The reduction of complex methylated formation arsenic species with the glutathione may be considered as a reason for the better translocation of methylated As species (Raab et al., 2007). Based on the tendency of uptake of As in rice to be: DMAA<arsenate< MMAA<arsenite (Marin et al., 1992; Raab et al., 2007).

3. Translocation of As

The accumulation and translocation of As in rice (grain and vegetative part) occur in metabolic pathways considering the order of translocation efficiency as DMA(V) > MMA(V) > inorganic As species (Kumarathilaka et al., 2018). Figure 1.7 shows the translocation mechanism of As in rice.

3.1. Translocation of inorganic As from root to shoot

Rice is the most efficient transporter where all major of inorganic (arsenate and arsenite) and organic As can be translocated from roots to shoots via the xylem. Figure 1.6 shows arsenic and arsenic species in root with iron plaque. According to the xylem analysis, oxidized As species as arsenate (86%) and DMA (14%) are dominant in the xylem. On the other hand, reduced arsenic species like arsenite (71%) and triglathione-AsGlu3 (29%) in vacuoles of cells adjacent to the xylem (Seyfferth et al., 2011). When inorganic arsenic species As (V) is transported from root to shoot via the xylem, in that time As (V) is translocated to the root cells via phosphate transporter (Smith et al., 2010; Punshun et al., 2017). As(V) is rapidly changed to As (III) after transport into the shoot cells, which can allow complex phytochelatins after sequestration into vacuoles (Carey et al., 2011; Raab et al., 2007; Zhao et al., 2010b). Phytochelatins are glutathione-derived heavy metal-binding peptides and phytochelatin synthesis is caused by heavy metalloids such as As. As (III)-phytochelatin complexes are thus sequestrated into vacuoles in root cells and to a lesser degree in stem and leaf cells, which are known to reduce the translocation of As to grains. Besides, Carey et al. (2011) suggested that As (III) is also transported to the shoot via xylem by complexation and sequestration in vacuoles in the roots. Once in the shoot, As (III) is taken up through aquaporins in leaf cells (Punshon et al., 2017). Researchers have already pointed out different arsenate reeducates which are the mutant gene of *OsHAC1;1*, *OsHAC1;2*, and *OsHAC1;4* are leading to decrease arsenate reduction in the root, lessen arsenite efflux, and increase arsenic accumulation in root and grain (Shi et al., 2016; Xu et al., 2017).

3.2. Translocation of organic As from root to shoot

However, depending on availability and abundance in soil, the amounts of organic As species have taken up by roots are much smaller, compare to inorganic As species. It is well known that organic forms of As are translocated more readily within the rice plant than their inorganic As counterparts (Raab et al., 2007; Li et al., 2009b; Carey et al., 2010). As a consequence of the altered molecular structure, the relative strong translocation of DMA to the shoot may be attributed, unlike As (III), to the weak SH (sulfhydryl) coordination (Raab et al., 2007). The key types of organic As, MMA, and DMA can therefore be translocated via xylem (Zhao et al., 2010b) into shoots. Then, in the shooting stage, leaf cells are taken up by aquaporins in the same way as As (III), MMA, and DMA (Zhao et al., 2010b). The findings, therefore, indicate in general that despite the pathways for accumulating roots and shot, As in its vacuoles is picked up and translocated into grains by the phytochelatin-associated As (III) complex. Arsenic translocated into grains depends significantly on the types and concentrations of As in soils, on the types and the rates of As absorbed by rice roots, the rice-growing ability to-reduce As (as absorbed into oxidized forms), which creates complexity with phytochelatins, sequester into cell vacuoles, and xylem flow volume.

3.3. Translocation of organic As to grain

A study of Carey et al. (2010) documented that shoot to grain translocation of As species in stem girdling found that phloem transport of As (III) and DMA accounted for 90% and 55%, respectively, in rice grain. This suggested that phloem is the primary route of transport to grains for As (III), while DMA is translocated via both phloem and xylem Carey et al. (2010). Several studies also found that a larger fraction of flag leaf DMA and

MMA is translocated to rice grains where As(V) is very poorly translocated and rapidly reduced to As(III) in flag leaves and As (III) displays no translocation (Norton et al., 2009, 2012; Carey et al., 2010). According to visible in distribution pattern, inorganic As present in the OVT region (Lombi et al., 2009) while DMA has been found in the endosperm of rice grain (Moore et al., 2010, Norton et al., 2010, Zheng et al., 2011). A survey of study from Sun et al. (2008) found that in brown rice, iAs is present in bran while endosperms contain much for DMA compare to other As species. Very recently, the *OsPTR7* gene has been identified in the root, leaf, and node 1 in the rice grain filling stage, which account for DMA in 35% of WT rice plants (Tang et al., 2017). Hence, *OsPTR7* is a long distance for root to shoot translocation and grain transport of DMA. Therefore, arsenic accumulation in rice grain, resulted mainly from phloem transport although it is unknown the translocation of As species out of the phloem (Punshon et al., 2017). Elevated As concentrations are following order: grain<husk<<leaf<<<root (Liu et al., 2004; Marin et al., 1992, Norton et al., 2012), a significant emphasis of arsenic levels in rice grain bran. It is noteworthy that to evaluate the arsenic concentration of rice layers in bran accounts for 23-29 percent of the total grain weight (Lombi et al., 2009, Moore et al., 2010, Sun et al., 2008). A synchrotron based X-ray fluorencencence microanalysis proved that arsenite was stored in the bran layer and ovular vascular trace (OVT) of a rice grain (Jackson and Punshon, 2015; Carey et al., 2011). Besides, increased DMA in the outer layers, followed by a higher concentration of DMA also present in the endosperm. Therefore, the risk of iAs is considered as the percent higher due to absorbing the ability of arsenic in rice grain.

4. Arsenic accumulation in rice grain

Arsenic accumulation in rice grain varies according to the genotype, farming systems, and environmental factors. Depending on geographical regions total As concentration may vary among different countries. For example, Norton et al. (2009) found that the significant largest variation in grain As and As speciation in three countries (Bangladesh, India, and China) was responsible for genotype and genotype-environment interaction. Even, the difference of As concentration in rice grain may differ in the same country due to the differences in environmental conditions, cultivar, and practical management. Ahmed et al. (2011) observed that As concentration was varied among the level of 38 cultivars grown at 10 sites in Bangladesh. Total As concentration varies among countries, which reaches up to fivefold, 20 fold differences. Rice produced in Asia and Africa, referred to a high portion of iAs, whereas rice produced in the USA, Australia, and Europe tends to have a low portion of iAs except for organic As. (Williams et al., 2005; Zavala et al., 2008). Smith et al. (2008) found that DMA and As(III) accounted for 85-94% and 6-15 % of the total As concentration in rice grain of Australia. Genotypic variation factors effect As accumulation in rice grain. Syu et al. (2015) estimated six types of genotypes deepening on the As content and species. They found that As concentrations were higher or equal than japonica cultivar among of those cultivars. Bhattacharya et al. (2013) investigated that hybrid rice varieties contain more As than local varieties in India and Bangladesh. More than 300 rice varieties are investigated based on laboratory screening to arsenite exposure, while showed significant variation in tolerance of genotypes along with a maximum of 13 fold difference in As accumulation (Dave et al., 2013). Hence, identifying genotype variation is an important step for rice cultivation and avoiding the cultivar of higher As accumulation in rice grains.

Depending on the process of a rice grain, arsenic contains differ in rice grain. It was reported that brown rice contains more arsenic than polished rice because its outer layers have a higher content of the metalloid (Meharg et al., 2008; Bakhat et al., 2017). Besides, Rice grains contain both inorganic and organic As species that can be varied according to the concentration: As(III)>As(V)>DMA>MMA (Huang et al., 2012; Meharg et al. 2008, Hu et al., 2015). Another finding of Zavala and Duxbury (2008), related to the color of the rice showed that arsenic concentration in brown rice 0.196 ± 0.111mg kg^{-1}, in white rice is 0.127 ± 0.087 mg kg^{-1} and 0.07 ± 0.05 mg kg^{-1} for other colors. Huang et al. (2012) proposed that compare to other As species, As (III) accounts for 90% of inorganic As collecting samples from at different sites of Asia using different types of treatment. Hu et al. (2015) found that the concentration of iAs and DMA were in 88% and 11% of total As in rice grain although rice grown aerobically. Moreover, organic and inorganic As species may differ according to the location of rice grain. For example, it also has been found that most of the As in the bran layers is present as oxidized As 69-88% in As(V) and 12-31% in DMA although As (III) was not detected in the endosperm in rice grain (Seyfferth et al., 2011) due to mobile nature of As species in rice grains. It is noteworthy that to evaluate the arsenic concentration of rice layers in bran accounts for 23-29 percent of the total grain weight (Lombi et al., 2009, Moore et al., 2010, Sun et al., 2008). A synchrotron-based X-ray fluorencencence microanalysis proved that arsenite was stored in the bran layer and ovular vascular trace (OVT) of a rice grain (Jackson and Punshon, 2015; Carey et al., 2011). Besides, increased DMA in the outer layers, followed by a higher concentration of DMA also present in the endosperm. Therefore, the risk of iAs is considered as the percent higher due to absorbing the ability of arsenic in rice grain.

5. Global warming affects the As concentration in rice grain

Predicted increase levels of As and global warming will have a serious amplify on the growth, yield, and quality of rice. Increased above of both critical issues will shorten the ripening period, occurrences to reduce carbohydrate accumulation in grain and thereby, low grain quality, and decrease the yield. These counteracting effects will determine the magnitude and even the direction of those impacts on human health. When the average daily air temperature 22–28 ◦C in the rice-growing season, the vegetative stage and grain filling stage are contributed to achieving high grain yield, and quality in rice (Deng et al., 2015). However, the rice organs panicle contributed to the grain weight decreased in the grain filling stage compare to the vegetative stage, while the optimum temperature exceeds due to the high temperature 40 days after heading (Kim et al., 1983; Morita et al., 2004). With an increase in the high temperature of 38 ◦C, rice plants particularly affected by a heat-wave during the development period of grain filling, terminate to tangible reduction scenarios lasting for 10-20 days in ripening time and concluded to vast estimated damage at total paddy yield loss of 5.18 million tons in China (Yang and Li, 2005; Tian et al., 2009). Kim et al. (2011) observed that high temperatures over 21 ◦C-shortened grain filling periods, which terminated to reduce the rate of grain filling in both phytotron and field experiments. They also suggested that the shortened grain-filling period is repulsed due to the earlier loss of sink activity rather than the earlier loss of the source of activity at high temperatures. Even, the early loss of sink activity damaged, resulting in a reduction of translocation ability and starch synthesis activity were included the reduction of cells on the dorsal side close to the vascular bundles under high temperature (Morita et al., 2005). Thus, high temperature accelerated the quality of grain and a great amount of yield. A study of Peng et al. (2004) described that rice grain yield declined by 10-15% for each 1°C daily mean air temperature increase in growing-season, while direct evidence associated with global warming.

Projected global warming is an unexpected warning, to have an enormous impact, has been reported high temperatures in recent years after rice heading could exacerbate the risk of increasing iAs accumulation levels in rice grains (Arao et al., 2018). From their multi-years (1995-2014) data analyzed provided consistency results that from 2 weeks after the heading day to 4 weeks after the heading day were significantly correlated with the inorganic As concentrations in the grains during the late-ripening period due to the increasing air temperature, although didn't the effect of inorganic concentration was observed in the early or late ripening stage. Muche et al. (2019) stated that climate-induced as over 33 ◦C high temperature changed in soil arsenic behavior, resulted in increasing inorganic As (iAs) in rice grain and decreased grain yield drastically. Hence, they proposed that combined effect of high temperature and increased soil arsenic resulted in a 42% decreased in yield with 81% grain filling, indicating pestered effect on the 21st century in the rice world.

Above that mention, increased iAs in rice grain through changing soil arsenic behavior by the high air temperature is becoming an important issue in the agriculture sector. However, several studies documented that high air temperature positively correlated with adjacent soil temperature until 20 cm depth of soil (Islam et al., 2015) and soil temperature plays an important role in aiding decision making for many processes like soil respiration, crop production, pest growth, germination, etc. (Ahmed, 2008). Increased soil temperature is one of the important factors for releasing As from soil or grown water (Tyrovola and Nikolaiods, 2009; Bonte et al., 2013). Moreover, the clear conception found that how elevated soil temperature effect to increase As bioavailability in the soil and increased the As concentration in the rice husk and straw (Neumann et al., 2017). Although they did not find the As concentration differs among their three-box treatments. In our recent studies focused that high air temperature had a possible relation to increasing soil temperature which turned to increase As concentration in rice grain and decreased grain yield that will be discussed in detail in chapter 2. Therefore, the rise in the As concentration in rice grains is not only due to the increase in soil temperature but also to the impact of the translocation to rice grains caused by the increase in air temperature.

6. The techniques of reduction of the arsenic in rice

6.1. Cooking method as a form of arsenic mitigation in rice

Considerably, cooking methods can immediate solution to reduce arsenic concentration in lowering the dietary exposure to As. Rinsing and cooking are the most recommended method in excess water discarded for reducing As in rice (Carey et al., 2015). Atiaga et al. (2020) carried in their study rinsing and boiling methods significantly reduce total arsenic compared with raw rice from Spain and Ecuador samples of rice. They also estimated that pre-rinsing could reduce lifetime health risk by 50% while combing it with discarding excess water can reduce the risk by 83%. Overall, the traditional method is practiced in southeast Asia, although the latter is commonly used in west Bengal. Kumarathilaka et al. (2019) found that cooking options could reduce the high amount of iAs concentration in raw rice grains from As contaminated areas that exceed 200 micro kg^{-1} of iAs. Hence, cooking rice in percolating water can reduce As concentration from As endemic areas. A survey of a study in Sengupta et al. (2006) proved in cooking unwashed rice in the 1:2 ratio refrain around 99.8%. Although, cooking can reduce As concentration in differing from Chinese and Hungarian rice, as 60% and 39% respectively (Mihucz et al., 2007). Carey et al. (2015) also found that cooking rice in a coffee making device decreased at 69% iAs.

6.2. Water management to reduce As concentration in rice grain

There are some mitigation processes to reduce As the concentration of rice grain. Among those processes, water management is effective to mitigate As concentration in the rice-growing season. Generally, aerobic and anaerobic have been followed to determine As accumulation in the whole rice-growing season. Besides, several studies have been focused on water management for partly flooding in rice, as like flooding to transplanting, after heading, 2-3 weeks, etc. Anaerobic conditions reduced plant height, tiller number, panicle number, panicle length, and grain yield than aerobic conditions (Shah et al., 2014). Even, before flowering rice plants uptake less As in aerobic conditions, which followed less As effect found in 13% grain, 94% straw, and 23% grain compared to plant subjected to anaerobic condition (Shah et al., 2014). Arao et al. (2009) investigated in their pot experiment to reduce As accumulation by 7 treatments of water management in rice-growing period and suggested that flooding in whole rice growing period is effective to increase As accumulation in grain compares to other treatments water management because of decreasing redox potential in the soil and oxidoreduction terminated arsenate to arsenite which has markedly solubility, plant availability, and toxicity (Takahashi et al., 2004). They also found that flooding for 3 weeks before and after heading considerably increased As and As species (DMA) concentration whereas aerobic treatment reduced those species concentration in rice grain. Flooding and draining during the rice-growing season, leading to large fluctuations in Eh, pH, and the solubility of As (Ishikawa et al., 2016). Typically, paddy water is drained during the late tillering stage to control excessive tillering, and during the mid-late grain filling stage for harvest. Under flooding conditions rice grown, some cases As concentration in grain can be increased 10-15 fold higher than aerobically rice (Xu et al., 2008). Moreover, alternate wetting and drying are also effective, in which the soil contained 40 to 60% of statured volumetric water content when the field was flooded reduced grain As concentration by

56% on average and improving water-use efficacy by 43-63% compared to the flooded treatment (Linquist et al., 2015).

6.3. Used low As cultivar to reduce As accumulation in rice grain

Although water management will not be always to reduce As concentration in rice grain, selecting low As cultivar may another way to survey As accumulation in rice cultivar. Low Cd rice cultivar `Koshihikari Kan No 1` may continuously decrease heavy toxic metals Cd and As in rice grain, if this cultivar grew AWD and WAS regimes (Ishikawa et al., 2016). However, depending on soil properties and water management, it may very difficult to continuously reduce Cd and As concentration in rice grain. Norton et al. (2009) proposed that some tropical japonica cultivars with low As have the potential to be used inbreeding. Spanu et al. (2012) demonstrated that 37 rice genotypes had only 2 % of the total grain As although those genotypes were grown in flooding. The aus variety Kasalath was to be more tolerant than the japonica variety Nipponbare because Kasalath took less arsenate As(V) than Nipponbare (Wang et al., 2016). However, low concentration of As varieties needs extended time for analyzing and developing in breeding.

6.4. Mitigation methods to use materials application to reduce As accumulation in rice grain

Due to variations in soil properties, such as microbial status, organic matter quality, iron oxides and minerals, and aggregate growth, the water management regime has induced various changes in the redox capacity of different soil types. Water control strategies intended to minimize concentration in different soil environments will not always be successful. Different agricultural steps were tested to minimize the transfer of arsenic to rice plants from soil or polluted water. Although, it is too hard to reduce As concentration in rice plants because the huge use is not of irrigation water is not strictly regulated in many countries (Xiao et al., 2018). Moreover, the ranking of rice cultivars for total grain As concentration varies greatly across environments (Norton et al., 2009), indicating the difficulty of genotype selection for lowering As in the rice grain and cost-effective. Water management, may affect rice yield and pathogen resistance, and selecting low As rice cultivars is quite time-consuming (Meharg, and Zhao, 2012). Therefore, different types of materials application (Si, Fe, etc) are effective to reduce As concentration in rice grain than above of those techniques. The concerns of Arsenic contamination have long been studied by scientists. Several measures have been mainly followed include remediation of the soil with soil amendments. Soil amendments improve the influence of arsenic by changing its speciation, decreasing the absorption or solubility of rice plants. Therefore, different types of materials application (Si, Fe, etc) are effective to reduce As concentration in rice grain than above of those techniques. However, Fe and Si materials are common application in paddy field farming to reduce As accumulation. Several reports of Fe band oxide increased into rice roots when applying Fe materials applied. Therefore, As is interrupted to accumulated in roots and suppression of the release of arsenite by the adsorption of arsenate by Fe. Thus, Fe materials reduce As concentration in rice grain although rice grown in As contaminated soils. On other hand, Si application is also applicable to prevent As concentration in rice grain because of chemical similarities between As and Si. Arsenic is transported by silicate in rice grain and rice has a high affinity to silicon. In our previous studies showed that Si materials could reduce As accumulation in rice grain which will be discussed in detail in chapter 4.

Several reports of Fe band oxide increased into rice roots when applying Fe materials applied. Moreover, As is interrupted to accumulated in roots and suppression of the release of arsenite by the adsorption of arsenate by Fe. Thus, Fe materials reduce As concentration in rice grain although rice grown in As contaminated soils. The application of iron oxides materials has been often recommended to reduce the impact of arsenic concentration in rice. It has been observed that Iron materials suppress impacts of changing soil pH, Eh and solubility of As in soil, tended to reduce arsenic concentration in rice grain (Kumpiene et al., 2008; Ultra et al., 2008; Suda and Makino, 2015, Matsumoto et al., 2015a, b; 2016). Ultra et al. (2009) added 0.1% and 0.5% amorphous iron oxides/hydroxides to the soil in a pot experiment associated with arsenic-contaminated water supply containing 5 mg /l arsenate which enhanced the formation of iron plaque around the root surfaces leading to a decreased As concentration in the rice plants and improved plant growth by increasing arsenic concentration on the root surface. Characteristics of iron plaque accumulation on mature rice plants and its impact on As (V) accumulation and speciation in the plants was studied by Liu et al. (2006) where distributed of arsenic in the plant parts was in the order iron plaque > root > straw > husk > grain.

Application of Si materials is well acknowledged to efficiently alleviate various chemical stresses caused by the toxicity of heavy metals like aluminum, cadmium, manganese, and iron. Some researchers have been focused that Si application is effective materials for healthy growth and development, improving tolerance to toxic metals toxicity, plant defense mechanisms produce an alteration in photosynthesis apparatus, simultaneously increases carboxylation, water, and light-use efficiency, etc. Due to the similarities chemical form of As and Si, Si significantly decreased elevated of As in the soil solution which followed by the stem, leaf, husk, and grain (Swedlundi et al., 1999, Waltham et al., 2002; Luxton et al., 2002). Recently, Li et al. (2018) found that Si significantly reduced the total As concentration in brown rice grains at the temperature range 22-35 ◦C. In our recent studies also showed Si materials significantly reduced As accumulation in brown rice grain.

7. Aims and objectives

There are several research articles on arsenic toxicity in rice grain and becoming the adverse health effect in taking iAs on their daily food of exposure to not only arsenic-contaminated soil but also arsenic uncontaminated soil. Most studies have shown that arsenic toxicity increasing in rice grain due to the As solubility increase in As contaminated soil. However, global warming is important to factor to increase As accumulation in rice grain, although recently focused that high temperature affects to increase iAs in rice grain between 2-4 weeks after heading using multi-years temperature (Arao et al., 2018). Moreover, they did not discuss specifically high-temperature effects As accumulation in rice grain. Therefore, we decided to research that high-temperature effect As accumulation and speciation in rice because of high temperature, and As of these effects is increasing globally. High As rich of rice would be reached in the market, amplify to health risk to humans through their taking daily food. Our research goal was to synthesize the daily increased high air temperature leading to an increase in arsenic concentration in rice grain.

1. The primary purpose of this study was to clarify high air temperature increased arsenic levels in the soil through increased adjacent soil temperature, resulted in more As accumulated in brown rice grain. This study hypothesized that high temperatures would have to increase arsenic levels in brown rice grain.
2. The second hypothesized that materials application (Fe, Si) may reduce As concentration in brown rice grain even in high-temperature conditions. This study also promotes a better understanding of arsenic toxicity in brown rice grain due to high temperature particularly for the populations that eat rice as a major source of food and temperature increases firstly.

3. The Third hypothesized that high temperature may reduce the dilution effect of carbon and interrupted functions of node, resulted to increase arsenic concentration in brown rice.

Table 1.1. Different sources of Arsenic in the environment.

Sources	Activities
Natural	Regolith originating from weathering and biological activity
Anthropological	Smelting, mining
Agricultural	Arsenical fertilizer, pesticides, herbicides, arsenic with livestock feed
Non-agricultural	Coal, petroleum, wood preservatives, electronics, industries, pharmaceutical works, galvanizing factories, ammonium factories

Collected from (Smith et al. 2008; Otles and Cagindi, 2010)

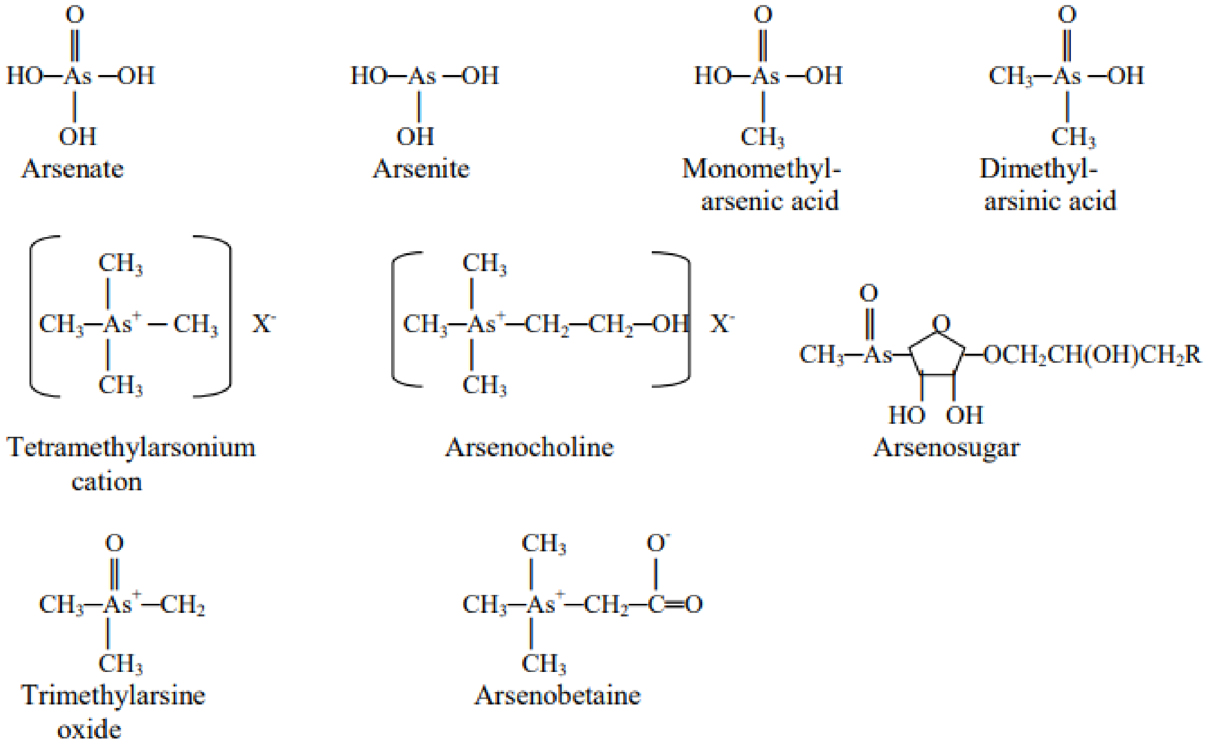

Figure 1.1. Arsenic species are found in soil, water, and plants (Adopted from Meharg and Hartley-Whitaker, 2002).

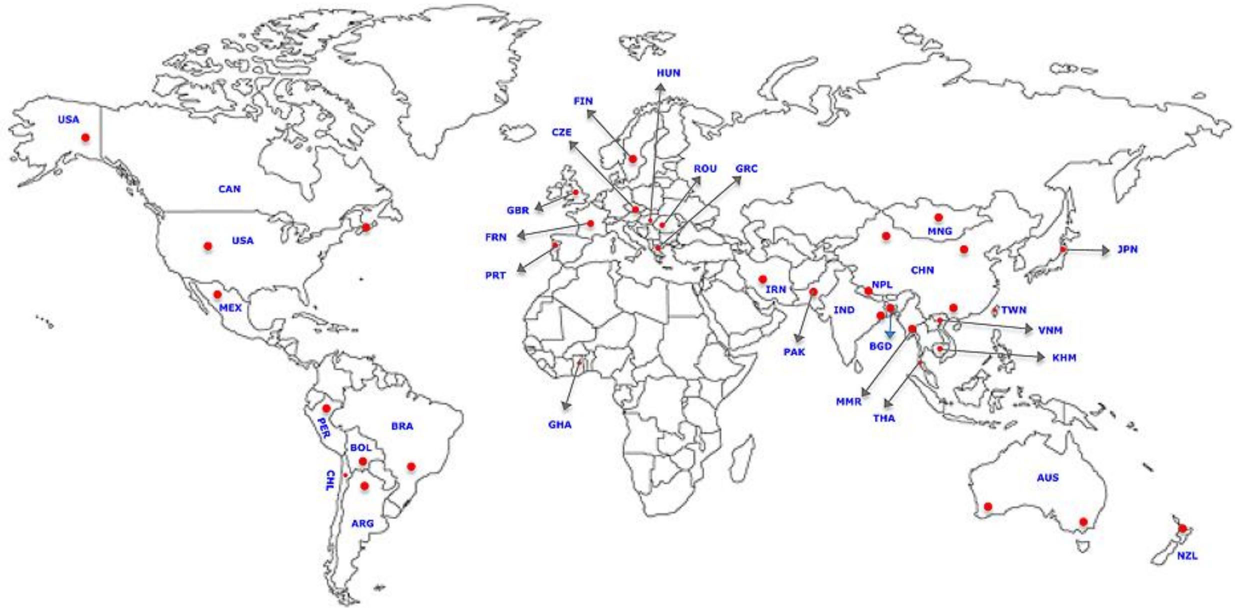

Figure 1.2. Arsenic contamination sites in the world. Adopted from Arsenocosis Global Health Crisis, InPhysics-Harvard University, by S. R. Sambu and Wilson, June 2008, Retrieved from http://wilson.physics.harvard.edu/home/map/.

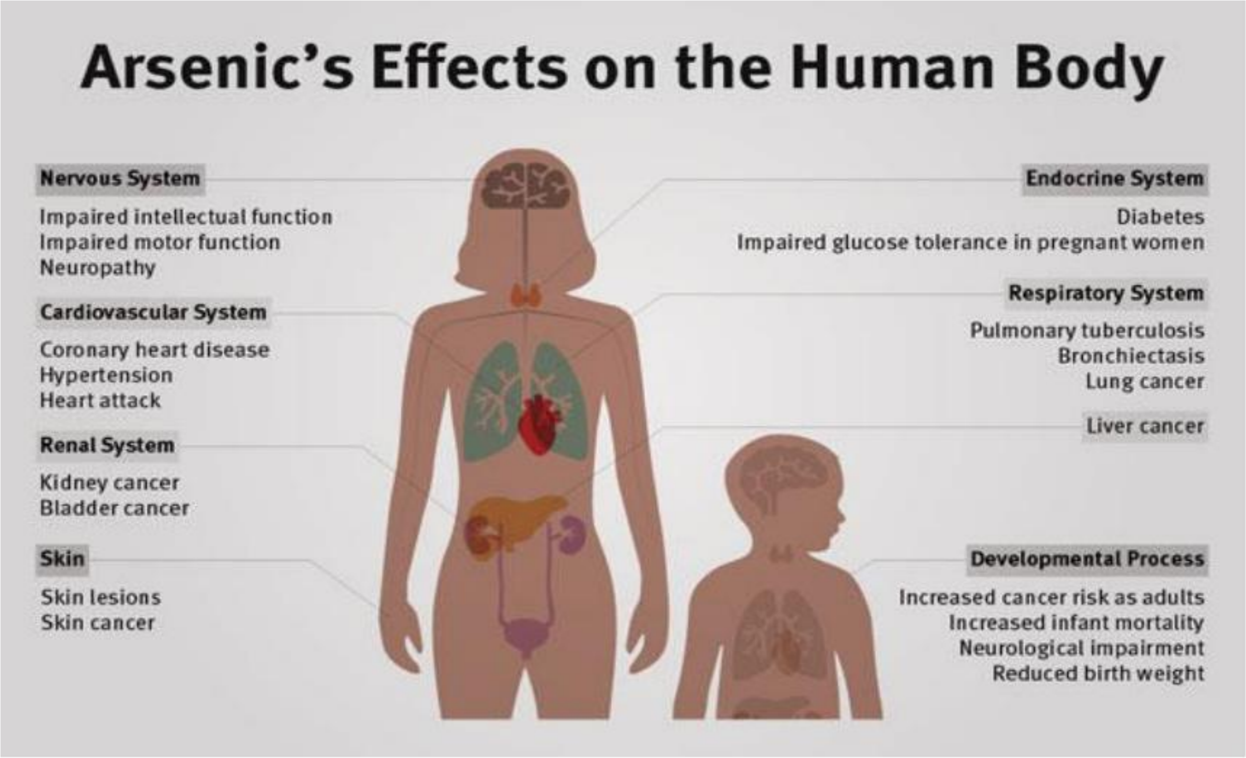

Figure 1.3. Arsenic affects the human health. Reprinted from Arsenic in Groundwater, In IASToppers, n.d., Retrieved March 20, 2017, from http://www.iastoppers.com/19th-20th-march-2017-current-affairsanalysis-iastopperst/. Copyright by IT's Current Affairs Analysis Team.

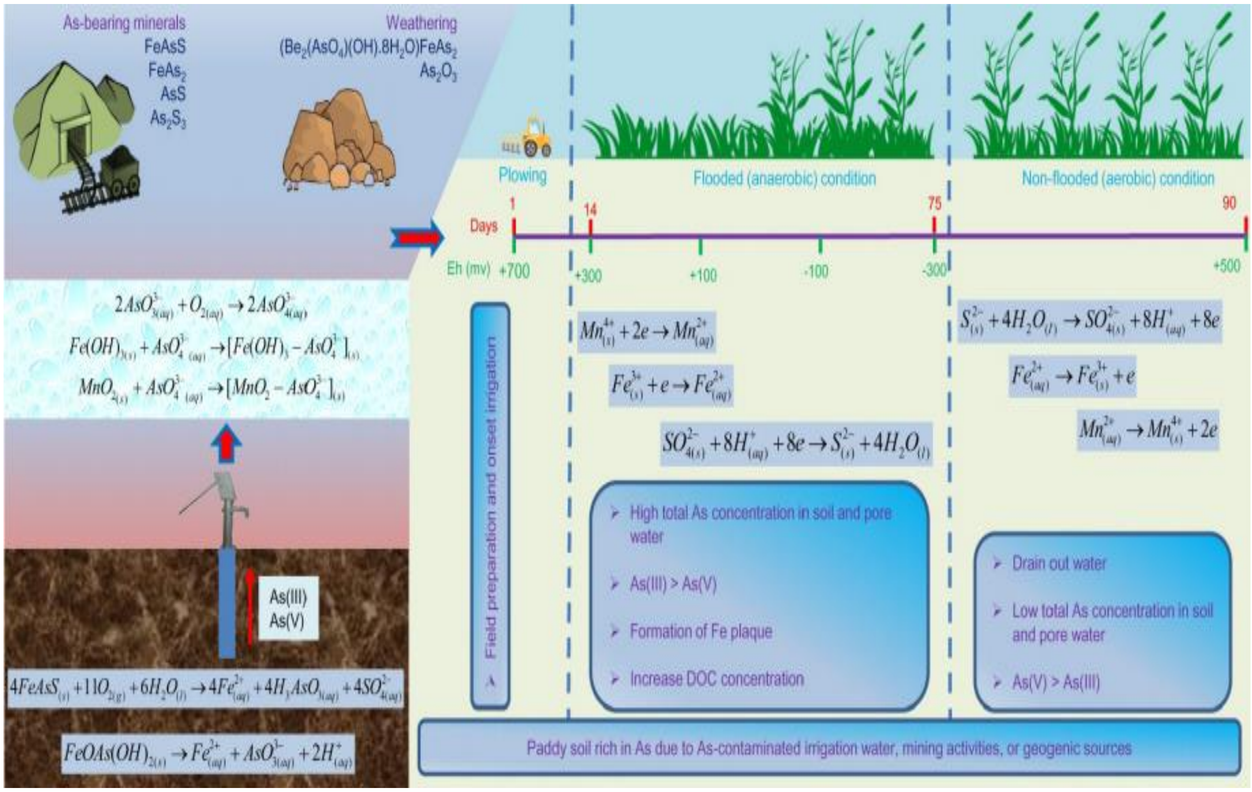

Figure 1.4. The mechanism of arsenic on arsenic-contaminated paddy soils (Adopted from Kumarathilaka et al. 2018)

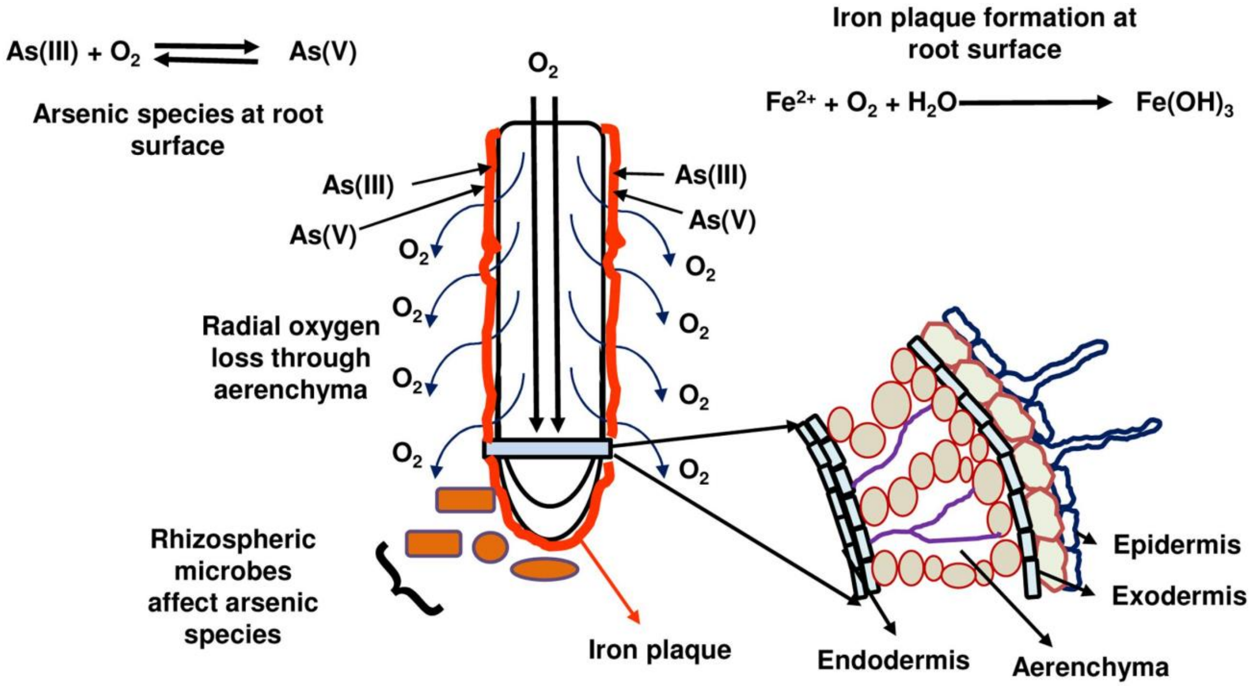

Figure 1.5. Arsenic availability on root surface and arsenic uptake by root (Adopted from Awasthi et al. 2017)

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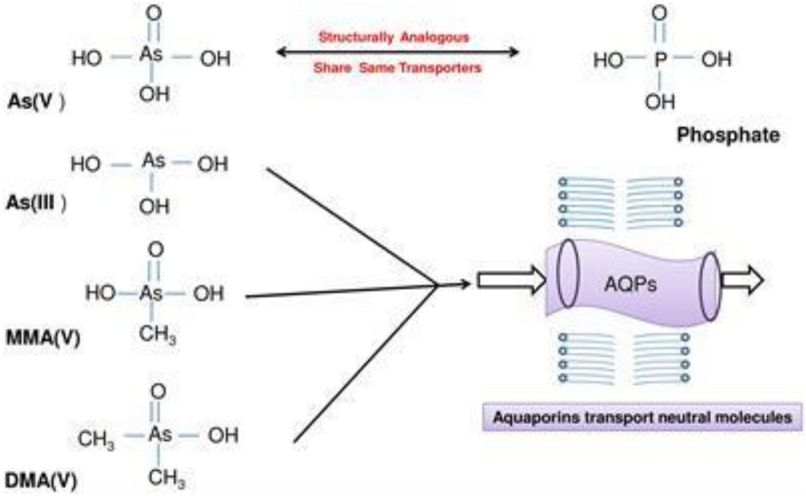

Figure 1.6 Arsenic (As) and As species tanslocation mechanism from root to shoot (Adopted from Awasthi et al. 2017)

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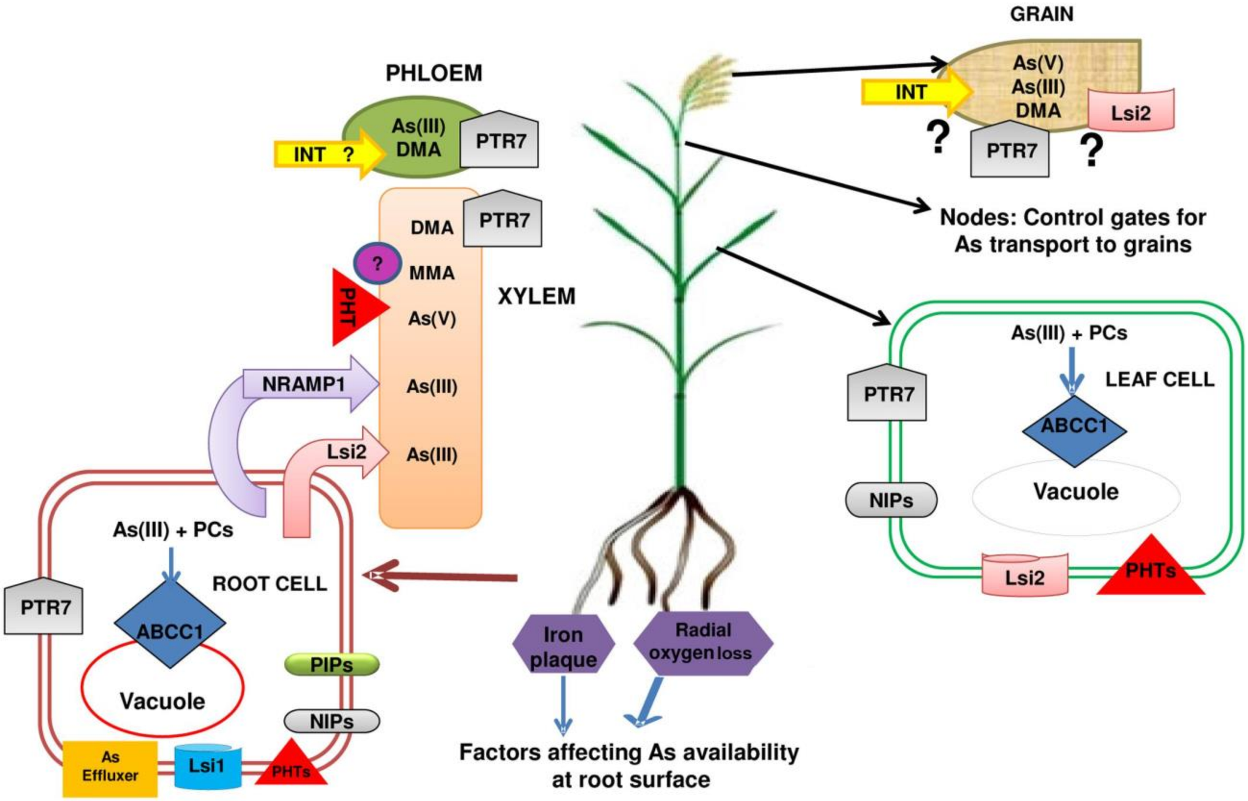

Figure 1.7. Arsenic translocation pathways from root to grain in rice (Adopted from Awasthi et al. 2017)

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Chapter 2

Effect of High Temperature during the Ripening Period on the Arsenic Accumulation in Rice Grain Grown on Uncontaminated Soil with Relatively Low Level of Arsenic

This chapter focuses on the present results of the assessment of the effect of high temperature on the arsenic concentration in rice grain one week after heading during the ripening period. This chapter also evaluated the present results of the daily air and soil temperature effect on As concentration in soil solution 1-6 weeks after heading. It also demonstrated the results of the effect of high temperature on the concentration As and As speciation in brown rice during the ripening period. Comparison of arsenic levels in rice yield from control and high-temperature treatments have been made in chapter two.

Abstract

The rice plants grown in Wagner pots (1/5000a) were cultivated in three TGCs set in three plots with control treatment, high-temperature treatment-1, and high-temperature treatment-2, respectively, from 1-week post-heading until harvest. We observed meaningful air and soil temperature differences between the control treatment and the two high-temperature treatments in the TGCs, but there was no clear temperature difference between the two high-temperature treatments. The concentration of total As in the soil solution during the ripening period was significantly higher in the high-temperature treatments than that in the control. The yield of brown rice in the high-temperature treatments was decreased by 17% via a decrease in the ripening rate compared to that of the control. The concentration of inorganic As (iAs) in brown rice grains was higher in the high-temperature treatments than in the control ($p = 0.065$). These findings suggest that the increase in the concentration of iAs in rice grains produced under a high-temperature condition may increase the risk of human As intake.

1. Introduction

According to the Intergovernmental Panel on Climate Change (IPCC) Fifth Assessment Report (IPCC, 2017), "It is very likely that human influence has been the dominant factor in global warming observed since the mid-20th century (over 95%)." It was also shown that there is no doubt about the continuation of such a high-temperature trend in the future, and there are significant concerns worldwide about the impact of high temperatures on agricultural products. In addition to the challenges presented by climate change, rice cultivation has faced difficulties due to the frequent occurrence of high-temperature ripening disorders that greatly reduce the grain yield and quality of rice (Morita, 2008; Morita et al., 2016). Peng et al. (2004) reported direct evidence that rice yields were reduced by nighttime temperature increases associated with global warming. At temperatures above the optimum growth temperature, dry matter production is hindered due to the small grain size. The higher air temperatures have caused poor maturation, low grain quality, sterility and finally, grain yield loss (Morita and Nakano, 2011).

Against this background of temperature changes due to global warming potentially leading to declines in rice yields and quality, Arao et al. (2018) analyzed the concentration of arsenic (As) in rice grain produced from 1995 to 2014. The concentration of inorganic As (iAs) in the rice grains showed a significant correlation with the average daily mean temperature (DMTs) between 2 weeks and 4 weeks after heading in the year when the rice was produced, and they suggested that the higher the temperature during the late-ripening stage of rice, the higher the iAs concentration in rice grain. Furthermore, Ministry of Agriculture, Forestry and Fisheries of Japan (2019), reported the results of a survey on the relationship between inorganic As (iAs) concentration in polished rice, physicochemical properties of soil, and meteorological factors from 3007 samples selected in proportion to the area of local governments nationwide from 2013 to 2016. In the survey, a multiple regression analysis was carried out with the iAs concentration in rice as the dependent variable, the soil characteristic value and the weather conditions after heading as the independent variables. Significant explanatory variables of this multiple regression model were selected for soil soluble As concentration, amorphous iron concentration, amorphous aluminum concentration, and DMT from 14 to 27 days after heading. Among these explanatory variables, DMT from 14 to 27 days had the highest standard partial regression coefficient, indicating high temperatures during the ripening period greatly contribute to increasing the iAs concentration in rice.

Arsenic is a non-essential, toxic metalloid that is naturally concentrated in rice grains. The accumulation of too much As in rice grains is a serious issue for both the yield and quality of rice (Abedin et al., 2002; Azad et al., 2012). Globally, the levels of toxic heavy metals — especially As — in rice have caused serious hazard exposures to human health, in a manner similar to that observed for chronic carcinogens (Panaullah et al., 2008; Meharg et al., 2009; Banerjee et al., 2013). Chronic As poisoning can cause serious health problems including cancers, hyperkeratoses, restrictive lung disease, and ischemic heart disease (Mandal and Suzuki, 2002; Rossman, 2003). It is thus very important to reduce the accumulation of arsenic in rice. We have shown that the addition of silicate and iron materials and intermittent irrigation can reduce arsenic concentration in brown rice (Matsumoto et al., 2015a; 2015b; 2016).

The Codex Alimentarius Commission (2016) has set the maximum allowable levels of inorganic As (iAs) in milled rice and brown rice at 0.20 and 0.35 mg kg^{-1}, respectively. It is thus required to reduce the As concentration in rice grown even in non-contaminated soil, and as noted above there is a concern that the As concentration in rice grains will increase due to the rise in temperatures globally.

Paddy rice contains greater As concentrations than other cereals crops (Williams et al., 2007) because it is grown mainly under flooded conditions (Meharg and Zhao, 2012). Arsenate (V) is strongly associated with soil mineral components such as Fe and Al (hydro) oxides (Goldberg, 2002), whereas arsenite (III) predominantly adsorbs to iron (hydro) oxides and is more mobile than arsenate. Generally, As is less mobile in aerobic conditions. Anaerobic conditions develop when there is a flooded reduction of soils, and reduction of soil by flooding leads to the reduction of the arsenate to arsenite (Yamaguchi et al., 2011; Ohtsuka et al., 2013), which is transferred by the silicic acid pathway in rice (Ma et al., 2008). Thus, As has markedly increased solubility, plant availability, and toxicity in anaerobic paddy fields (Takahashi et al., 2004).

Although Arao et al. (2018) indicated that high temperatures after heading could increase the As concentration in rice grains, it has not yet been determined whether high-temperature treatment would change the solubility of As in the soil and/or increase the As concentration in rice grains. A temperature gradient chamber (TGC) was designed to provide appropriate techniques to develop experimental facilities for research on temperature stress in rice and the responses of rice to global environmental changes (Okada et al., 1995). A TGC is an extremely effective system for studying the effects of temperature in crop cultivation because it enables high-temperature treatment linked to the actual temperature. Since it is necessary to study and identify the effects of temperature on rice grains' As accumulation and As speciation for future paddy rice cultivation where temperature rise is expected, we decided to use a TGC to investigate the effects of temperature change after heading on the solubility of As from soil and the concentration of As in rice grain. We conducted the present study to determine the As uptake in rice grains and to identify the relationship between temperature and the As concentration in rice grains during the ripening period. We hypothesized that with a large increase in temperature after heading, it would be possible to spontaneously increase the As concentrations in the rice grains. In conducting this experiment, we tested uncontaminated soil with relatively low As concentration. Because rice cultivated on contaminated soil or soil with extremely high As concentration is very unlikely to reach the market, so we think it is better to conduct experiments using soil where the As concentration of the soil is not so high. It is suitable for the evaluation of food production and distribution and food safety.

2. Materials and Methods

2.1. Rice cultivation

Selected good-quality sterilized seeds of 100 g of rice (*Oryza sativa* L. cv. Koshihikari) were germinated at 32 °C for 24 h. Germinated seeds were sown in paper pots (comprised with 578 blocks of 1.5 cm square and 3 cm depth). For rice cultivation, we used Wagner pots (1/5000 a, Fujiwara Scientific, Tokyo) filled with 2.5 kg of uncontaminated gray lowland soil collected from the plowed layer of a paddy field in the Honjo experimental farm of Shimane University, Matsue, Japan (35º51′N, 133º11′E, 4 m asl), and we mixed 2.8 g of a compound fertilizer in every pot so that each pot contained N 14%, P_2O_5 14%, and K_2O 14% by basal application before the seedlings were transplanted. The tested soil contained 1.6% total C, 0.14% total N, and 1.0 mg kg^{-1} of 0.1 M HCl extractable As (i.e., available As), and it had a pH of 5.5.

Approximately 25-days-old seedlings were transplanted to the Wagner pots on May 31, 2018. Three seedlings were transplanted in the center of each pot. Every pot was maintained in flooding conditions until harvesting. We applied liquid fertilizer which contains 0.21 % N as a form of $(NH_4)_2SO_4$ per pot with 0.5 g of N at 48 days after the seedlings were transplanted. The plants were grown outdoors before the start of high-temperature treatment.

2.2. High-temperature treatment during the ripening period

To evaluate the As accumulation in rice grains under high-temperature conditions, we used three 15-m-long TGCs (Fig. 2.1) (Okada et al., 1995). The TGC experiments were performed in 2018 in a field at Shimane University, Matsue, Japan (35º29′N, 133º04′E, 4 m asl). Three temperature regimens were applied in order from the entrance of the TGC to deep inside the TGC: control treatment, high-temperature treatment-1 (HTT-1), and high-temperature treatment-2 (HTT-2). The boundaries of each treatment section were separated by a transparent nylon curtain with vertical slits. Each TGC was equipped with a fan on the backside, which creates a gentle airflow from the entrance to deep inside. For this reason, the temperature in control is close to the outside temperature due to the airflow passing through the entrance, and the temperature gradually increases toward the deep inside (Fig. 2.2). The TGC was designed to provide temperature increases from the entrance to the deep inside, so that the air and soil temperatures are expected to be in the following order: control < HTT-1 < HTT-2. The daytime air temperature was set to not exceed 40°C by adjusting the fan exhaust speed.

The heading date was August 3rd when 50% of the total heading occurred. After seven days from the heading date (August 10th), three pots were transferred to each of the temperature treatments in each of the three TGCs and grown in the TGCs until harvesting (September 19th). The air temperatures and soil temperatures were recorded until harvesting.

2.3. Soil solution sampling

To collect the soil solution, a soil water sampler (# DIK8393, Daiki Rika Kogyo, Saitama, Japan) was buried at a depth of 5 cm in the soil of one of the three pots in each section of each TGC 7 days before heading. The soil solutions were sampled at days 7, 14, 21, 28 and 41 after heading. The sampled soil solution was immediately mixed with 10% HNO_3 in a 9 (soil solution): 1 (10% HNO_3) ratio to prevent precipitation due to iron oxidation (Ma et al., 2008).

2.4. Plant sampling

At maturity (September 19th), we harvested rice plant samples from each pot, and the harvested samples were air-dried in a greenhouse for 7 days. The air-dried samples were divided into the ears and straw. Husks and unfilled grains were removed. The panicles were divided into rachis and grain. Unfilled grains were removed by ammonium sulfate solution, and the grains were counted by a machine (#IC-1, Aidex Co., Nagoya, Japan). Filled grains were de-husked by a machine (#FC2K, Otake Agricultural Machinery Co., Aichi, Japan).

2.5. The determination of As in brown rice and the soil solution

Five ml of 5: 1 (v / v) HNO_3 / H_2O_2 was added to a powdered sample of brown rice (0.5 g), and the samples were wet digested in a microwave oven (#MLS 1200, Milestone, Bergamo, Italy). The concentrations of As in the degraded samples and the soil solutions were determined by inductively coupled plasma mass spectrometry (ICP-MS) (#ELAN DRC-e, PerkinElmer Sciex, Shelton, CT).

We determined As speciation in rice grains by using the method of Arao et al. (2011). Powdered brown rice (0.5 g) was mixed with 2 mL of HNO_3 (0.15 M) in a 10 mL capped high-density polyethylene centrifuge tube, and the mixture was heated in an aluminum heating block at 80 °C for 2 hours. The obtained extract was diluted to 10 ml with water and passed through a 0.45 μm filter before analysis. As-speciation of diluted solution samples was determined using high-performance liquid chromatography (HPLC) / ICP-MS system. A Super IC-Anion column (5 μm ID, 4.6 mm ID, 150 mm ID) and a guard column (Tosoh, Tokyo) equipped with an isocratic mobile phase system consisting of 10 mM ammonium acetate and an HPLC system (#PU 712i, GL Sciences, Tokyo) were used. Injection volume and mobile phase flow were set at 10 and 800 $\mu Lmin^{-1}$, respectively. Arsenic concentration was determined using the ELAN DRC-e ICP-MS system.

Total inorganic As is expressed as the sum of arsenite and arsenate. The accuracy of the analysis was certified by the reference material (CRM) (rice flour, NMIJ CRM 7503-a: arsenite 0.0711 ± 0.0029 mg kg^{-1}, arsenite 0.0130 ± 0.0009 mg kg^{-1}, dimethyl arsenate (DMA) 0.0133 ± 0.0009 mg kg^{-1}; National Institute of Advanced Industrial Science and Technology [NIJM], Japan). According to the NIJM CRM 7503-a guidelines, the values for arsenite, arsenite, and DMA were determined as 0.082 ± 0.003 mg kg^{-1}, 0.022 ± 0.006 mg kg^{-1}, and 0.018 ± 0.001 mg kg^{-1}, respectively.

2.6. Statistical analyses

The data were analyzed using a repeated measure analysis of variance (ANOVA) for the soil solution. A two-way ANOVA without repetition was performed with TGC as a block factor and temperature treatment as a control factor for the analysis of growth, yield and As concentration of brown rice, using the mean values of three pots placed in each temperature treatment in each TGC as representative values. When the ANOVA showed significance, the multiple comparisons were performed by Tukey's HSD test.

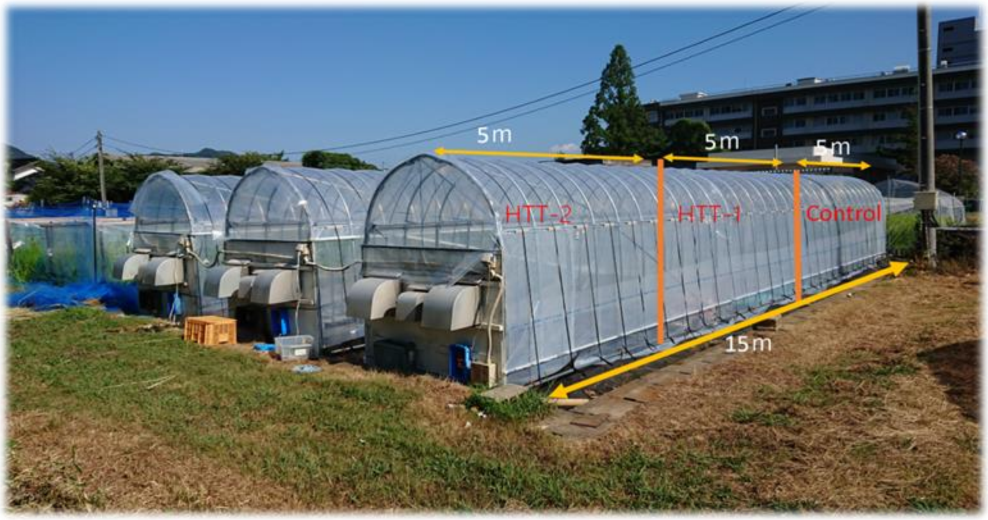


Figure 2.1. Three Temperature Gradient Chamber (TGCs) used for high-temperature treatment during the ripening period. Here HTT-1 and HTT-2 defined as high-temperature treatment 1, and high-temperature treatment 2, respectively.

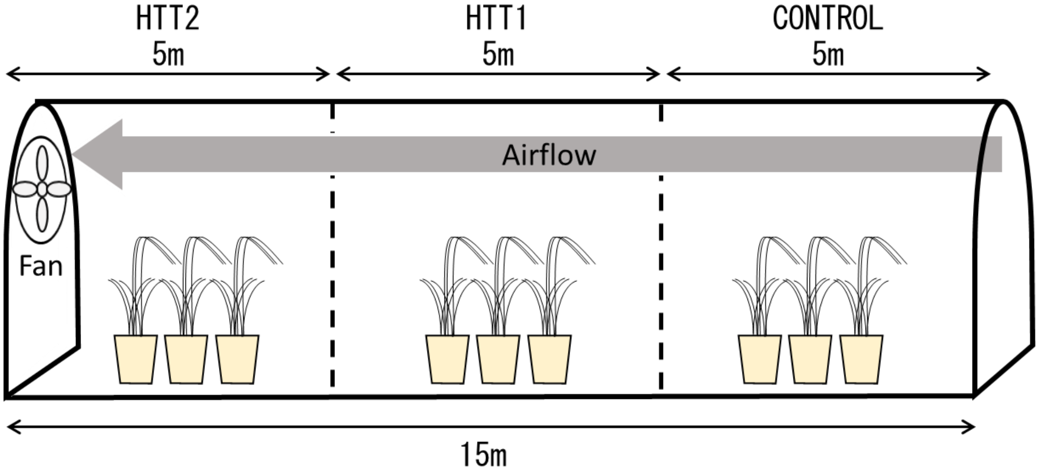


Figure. 2.2. Schematic inside of TGC

3. Results

3.1. Temperature differences between the control, HTT-1, and HTT-2 in the TGCs during the ripening period

Fig. 2.3 a–c shows the daily air temperature variation in the control, HTT-1, and HTT-2 for T_{mean}, $T_{max,}$ and T_{min} in the TGCs during the ripening period. The differences in the average daily air temperatures among the three treatments (control, HTT-1, and HTT-2) were 0.02–1.98 °C (T_{mean}), 0.30–3.70 °C (T_{max}), and 0.10–1.93 °C (T_{min}). The average daily mean air temperature during the ripening period was 26.9 °C in the control, 27.9 °C in the HTT-1, and 27.6 °C in the HTT-2 (Table 2.1). The average daily mean temperature changes were within 1.0 °C during the ripening period. The average daily air maximum temperature was 33.6 °C in the control, 34.6 °C in the HTT-1, and 34.0 °C in the HTT-2; as a result, the average daily maximum temperature changes were within 1.0 °C during the ripening period among the three temperature treatments.

On the other hand, the average daily air minimum temperature was 22.6 °C in the control, 23.7 °C in the HTT-1, and 23.6 °C in the HTT-2 during the ripening period (Table 2.1). Therefore, the average daily air minimum temperature changes were within 1.1°C during the ripening period. We observed a meaningful air temperature difference between the control and the two high-temperature treatments by using TGCs. However, the air temperature difference between HTT-1 and HTT-2 was small, and in contrast to the setting, HTT-1 was slightly higher than HTT-2.

The daily soil temperature differences are shown in Fig. 2.4 (a–c) for the control, HTT-1, and HTT-2 in the TGCs for T_{mean}, $T_{max,}$ and T_{min}. The variations in the daily soil temperature differences among the control, HTT-1, and HTT-2 treatments were 0.00–2.50°C (T_{mean}), 0.05–2.30°C (T_{max}), and 0.57–3.13°C (T_{min}). The average daily soil temperature differences for T_{mean} and T_{max} between the control and HTT-1 differed slightly among the three TGCs. The range of temperature differences for T_{min} was greater than that for T_{max} during the ripening period.

The average daily soil mean temperature during the ripening period was 26.9 °C in the control, 27.5 °C in the HTT-1, and 28.0 °C in the HTT-2 (Table 2.1). The average daily soil maximum temperature during the ripening period was 29.5 °C in the control and 30.5 °C in the HTT-2. The average daily soil minimum temperature during the ripening period was 24.9 °C in the control and 26.0 °C in the HTT-2. The differences in the average daily mean, maximum and minimum temperatures were within 1.0- 1.1 °C during the ripening period. Based on this soil temperature data, we detected an effective soil temperature difference among the temperature treatments. However, the differences in the air temperature and soil temperature between HTT-1 and HTT-2 were close compared to those between the control and the two high-temperature treatments in the present study. The reason why the expected temperature difference did not occur between the two high-temperature treatments may be that 2018, when this study was carried out, was a high-temperature year. The average temperature in Matsue city during the high-temperature treatment was 0.8 °C higher than the normal temperature. In the TGC used in this experiment, to prevent excessive high temperature, the fan was set to operate when the temperature in the HTT-2 section exceeded 40 °C. The difference between the high-temperature treatments tended to be considered to be less likely to occur when the outside air temperature was high. Moreover, the short length of the TGC used in this study (15 m) may be one reason why there was no difference in temperature between the two high-temperature treatments. Therefore, we decided to analyze the data from these two treatments (HTT-1 and HTT-2) as one high-temperature treatment for the analysis of soil solution, growth of rice, and As concentration of brown rice.

3.2. The effects of high temperature on the total As concentration in soil solution during the ripening period

We used a repeated measures ANOVA to analyze the concentration of total As in the soil solution during the ripening period (Table 2.2). The ANOVA validated that the total As concentrations had a significant effect on the As concentration in the soil solution under the temperature treatment. The average total As concentration in the soil solution during the ripening period was 48.1 μg L^{-1} in the control but significantly increased in the high temperature treatment (55.3 μg L^{-1}). The sampling dates also showed significant changes within the ripening period. The arsenic concentrations in the middle ripening stage (14 to 28 days after heading) tended to be higher than those in the early stage (7 days after heading) and the harvesting period (41 days after heading). That is, the As concentration in the soil solution tended to increase in the midsummer. These results also indicate that the high temperatures increased the total As concentration in the soil solution during the ripening period.

The changes during the ripening period in both treatments were shown in Fig. 2.5. The total As concentration in the soil solution in the high-temperature treatment was consistently higher than that in control during the treatment. The high temperature thus effectively increased the As concentration in the soil solution.

3.3. The effects of high temperature on the brown rice yield and yield components

The brown rice grain yield and yield components are provided in Table 2.3. The high temperature during the ripening period tended to reduce the ripening percentage. In contrast, the thousand-grain weight tended to be increased by the high-temperature treatment. However, the brown rice grain yield was decreased by 17% compared to that of the control though the difference was not significant.

3.4. The effects of high temperature on the As concentration and As species in the brown rice

The results of the statistical analyses of the As concentration and As species in the brown rice are summarized in Table 2.4. Although there was no significant difference, the total As concentration in brown rice tended to be higher in the high-temperature treatment than in control. The concentrations of DMA and monomethylarsonic acid (MMA) did not show significant differences between the high-temperature treatment and control. In contrast, iAs concentration in brown rice showed a meaningful difference between high-temperature treatment and control though a significant difference was not detected at a 5 % level of significance. The concentration of iAs in the high-temperature treatment was higher than that of the control (p = 0.065). Therefore, increasing of total As in brown rice mainly depends on the increasing of iAs.

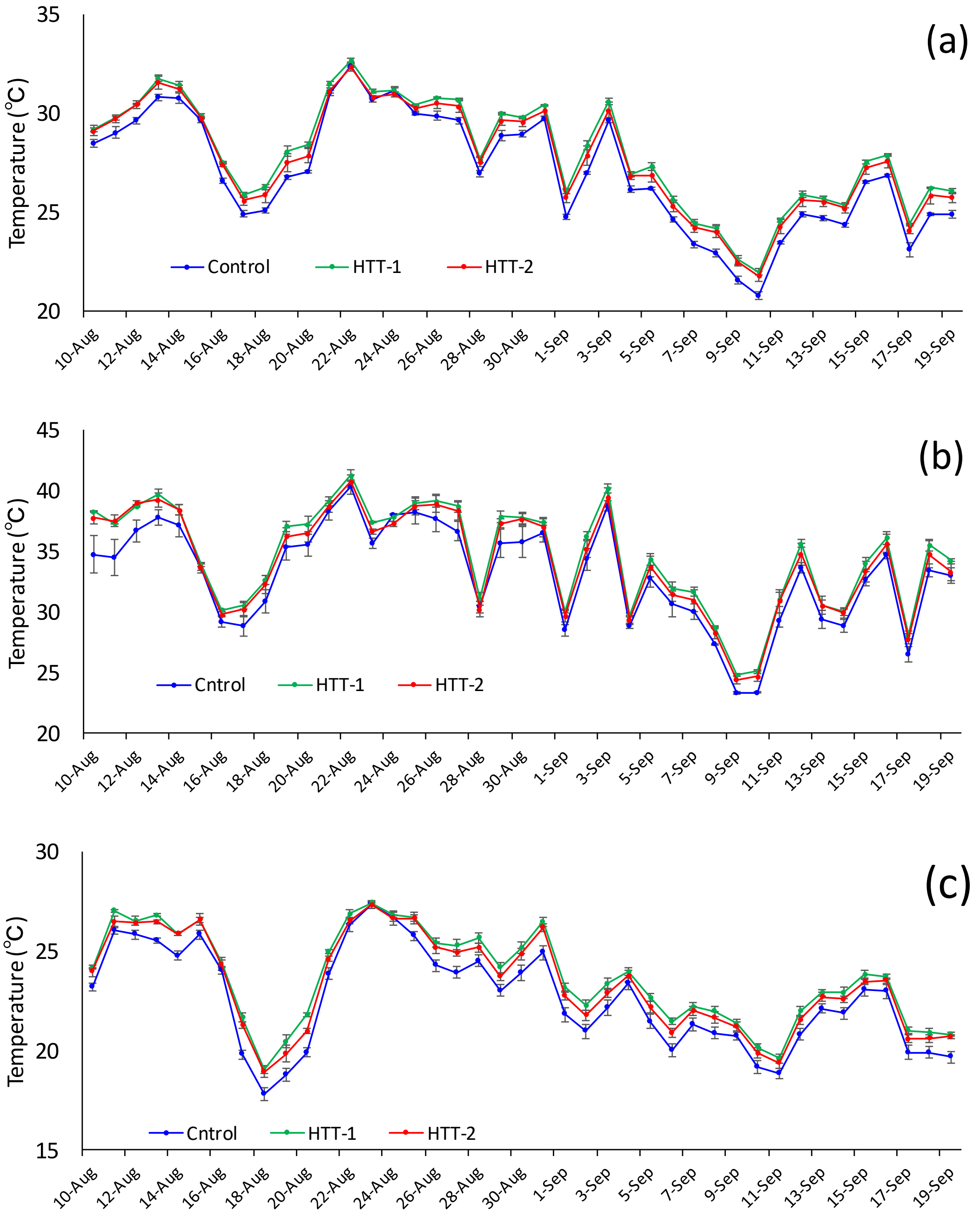


Figure. 2.3. Changes in average daily air mean temperature (a), daily air maximum temperature (b), and daily air minimum temperature (c) during the ripening period. HTT-1, high-temperature treatment 1; HTT-2, high-temperature treatment 2. Mean ± se (n = 3).

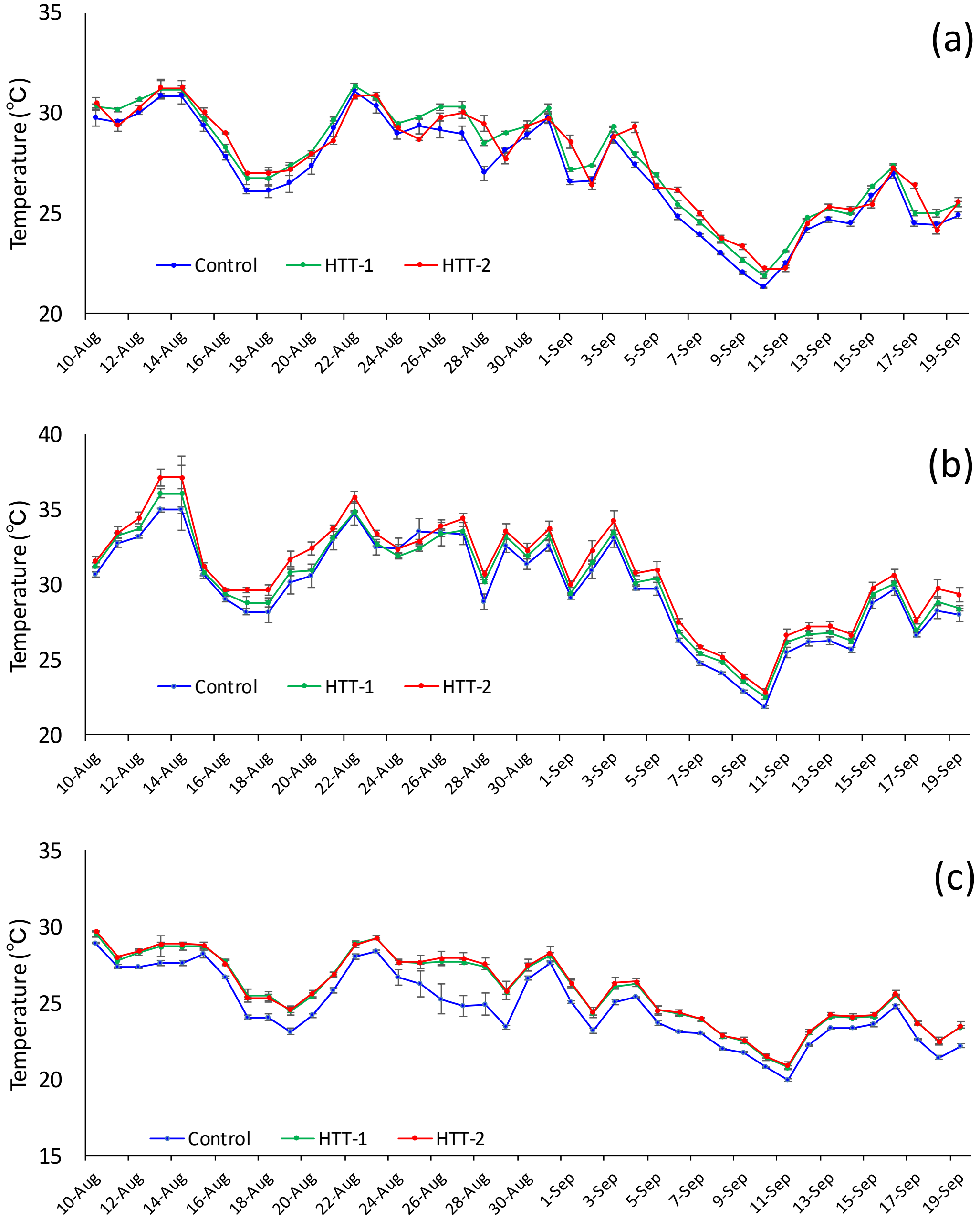


Figure. 2.4. Changes in average daily soil mean temperature (a), daily soil maximum temperature (b), and daily soil minimum temperature (c) during the ripening period. HTT-1, high-temperature treatment 1; HTT-2, high-temperature treatment 2. Mean ± se (n = 3).

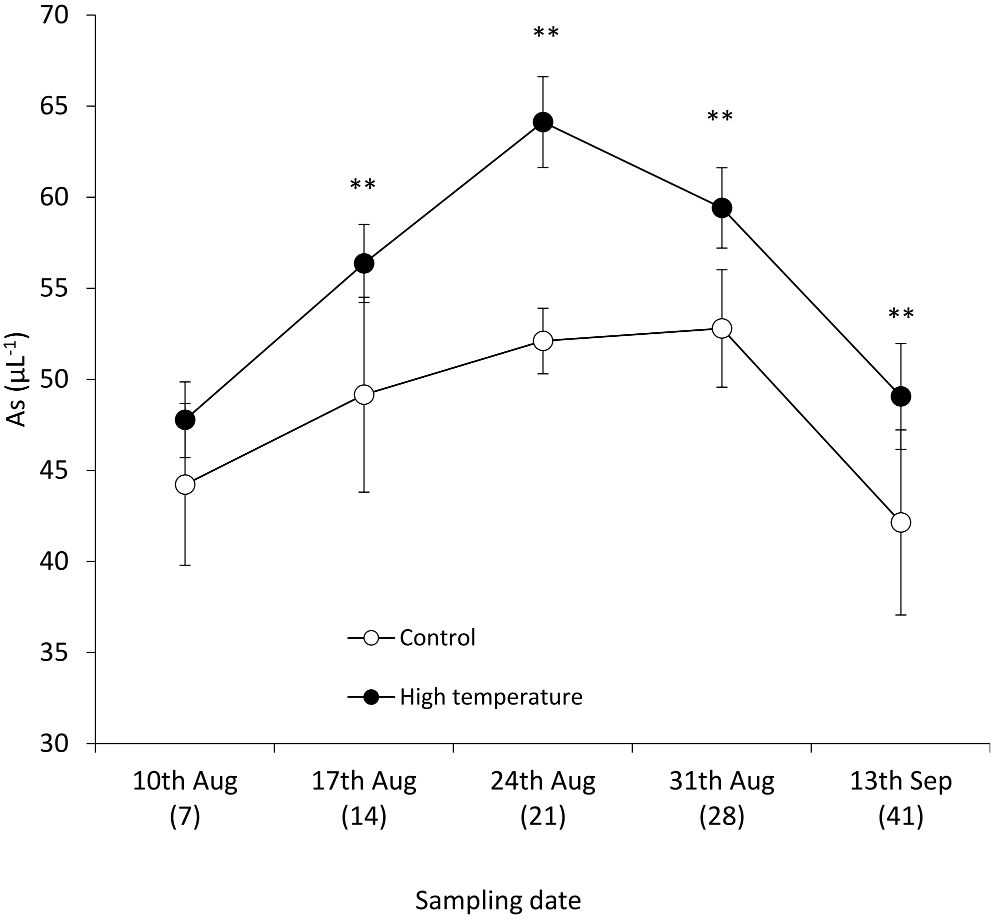


Figure.2.5. Changes in the concentration of total As in soil solution during ripening period.
Mean ± se (n = 3).
** Represents significance at the 0.01 probability level.
Numbers in parentheses indicate days after heading.

Table 2.1. Average air and soil temperature during ripening period.

	Air temperature (℃)			Soil temperature (℃)		
	Mean	Maximum	Minimum	Mean	Maximum	Minimum
Control	26.9 ± 0.1	30.6 ± 1.0	22.6 ± 0.5	26.9 ± 0.2	29.5 ± 0.6	24.9 ± 0.1
HTT-1	27.9 ± 0.2	34.6 ± 0.5	23.7 ± 0.2	27.5 ± 0.1	30.0 ± 0.1	25.5 ± 0.1
HTT-2	27.6 ± 0.5	34.0 ± 1.1	23.6 ± 0.3	28.0 ± 0.2	30.5 ± 0.5	26.0 ± 0.3

Mean ± se (n = 3)

Table 2.2. Effect of the temperature and sampling date on the concentrations of As in soil solution

Main factor	Treatment	Total As (mg L^{-1})
Treatment (T)	Control	48.1±1.9 b
	High temperature	55.3±1.9 a
Sampling date (D)	10th August (7)	46.6±2.3 B
	17th August (14)	54.0±3.0 AB
	24th August (21)	60.1±3.0 A
	31th August (28)	55.0±3.4 b
	13th September (41)	46.8±3.0 B
Analysis of variance	T	$p = 0.011$ *
	D	$p = 0.008$ **
	T x D	$p = 0.834$

Mean ± se (temperature, n = 15; sampling date, n = 6).

*Represents significance at the 0.05 probability level.

** Represents significance at the 0.01 probability level.

Different letters within a column indicate significant difference by Tukey's HSD test ($p < 0.05$)

Numbers in parentheses indicate days after heading.

Table 2.3. Effect of the temperature on brown rice yield and yield components.

Main factor	Treatment	Panicle number (Pot^{-1})	Grain number (Panicle^{-1})	Ripening percentage (%)	Thousand grain weight (g)	Brown rice yield (g Pot^{-1})
Temperature (T)	Control	21.8±1.5	73.6±2.9	81.4±0.0	23.2±0.3	30.1±1.0
	High temperature	22.2±0.2	71.4 ±1.3	66.6±0.1	24.2±0.3	25.1±1.7
Analysis of variance	T	$p = 0.606$	$p = 0.628$	$p = 0.144$	$p = 0.375$	$p = 0.157$
	TGC	$p = 0.071$	$p = 0.765$	$p = 0.544$	$p = 0.980$	$p = 0.691$

Mean ± se (n = 3)

Thousand grain weight and brown rice yield converted to a moisture content of 15%.

Table 2.4. Effect of the temperature during ripening period on the concentrations of As species in brown rice at harvest.

Main factor	Treatment	Total As (mg kg^{-1})	Inorganic As (mg kg^{-1})	DMA (mg kg^{-1})	MMA (mg kg^{-1})
Temperature (T)	Control	0.309±0.019	0.219±0.005	0.068±0.017	0.006±0.001
	High temperature	0.323±0.012	0.234±0.009	0.064±0.008	0.006±0.000
Analysis of variance	T	$p = 0.225$	$p = 0.065$	$p = 0.695$	$p = 0.866$
	TGC	$p = 0.064$	$p = 0.071$	$p = 0.125$	$p = 0.372$

Mean ± se (n = 3)
The concentration is a value converted to a moisture content of 15%.

4. Discussion

4.1. The relationship between temperature and the dissolution of As in soil

High temperatures are a serious issue in agricultural, in part because they have a great impact on the increase in the bioavailability of As fixed in the soil's solid phase by the activation of microbial activity (Neumann et al., 2017). In anaerobic soil or flooded paddy soil, the form of As changes depending on the oxide reduction in the soil (Takahashi et al., 2004). Because of the reduction of As from As(V) to As(III) during the flooded period, As(III) is quickly released from soil to water, and then the solubility, toxicity, and bioavailability of As to rice are enhanced (Takahashi et al., 2004).

Rice roots can oxidize the reductive dissolution of As bearing the host Fe (II) oxyhydroxide minerals that scavenge As near or on roots (Hu et al., 2005; Frommer et al., 2011; Seyfferth et al., 2010; Williams et al., 2014). The As is then transferred to the grains and stored mostly in the bran layer (Lombi et al., 2009; Carey et al., 2010; Seyfferth et al., 2011). Some of the microbial bacteria are responsible for releasing As; they are controlled by the rhizosphere process (Weber et al., 2010; Jia et al., 2014). The dynamics of dissolved Fe and As in a soil solution were reported to depend on the temperature, as dissolved Fe and As were rapidly increased and stabilized at a rate of 5.5 mM Fe and 110 mM As when the soil temperature was increased from 10 °C to over 20°C in a contaminated floodplain soil (Weber et al., 2010).

Tyrovola and Nikolaidis (2009) reported that the release of As from contaminated topsoils is related to temperature. The concentration of As was higher at 40°C than the equilibrium concentrations at 10 °C and 20 °C in contaminated soil, and a higher concentration of arsenate was released as the soil temperature rose. Based on their report, we suspect that the temperature could have affected the release of the different As species from the soil at different temperatures (10°, 20°, and 40°C). Thus, the release of As from the soil's solid phase depends on the temperature through the activity of iron-reducing bacteria, which can reduce insoluble ferric oxide in aquifer soils to soluble ferrous hydroxide and use the oxygen released by that change to oxidize some of the remaining organic material, providing a strong influence to release toxic metals in the soil (Gounou et al., 2010).

Neumann et al. (2017) cultivated rice plants in California paddy soil packed into rhizome boxes, using different soil temperatures. In two soil temperature treatments (26.1 °C and 30.5 °C), they used plant stems for the synchrotron X-ray fluorescence (XRF) imaging of solid-phase As and Fe after heading. Interestingly, the XRF imaging at the root base of the rice plants showed that more Fe and As were accumulated at 30.5 °C compared to the 26.1 °C soil temperature treatment. This is because soil high temperature is related to increased rates of microbial activities mediated by the reductive dissolution of As bearing iron oxides, releasing comparatively more As from the soil. Increasing soil temperature could therefore affect the increases in the solubility and toxicity of As accumulated in rice plants.

The results of our present TGC experiments agreed with those of above-cited studies. We observed that the average mean soil temperature was 26.9 °C in the control, 27.5 °C and 28.0 °C in the high-temperature treatments (Table 2.1). The high-temperature treatments during the ripening period significantly increased the As concentration in the soil solution compared to that of the control treatment. The As concentrations in the soil solution in the high-temperature treatment remained higher than that in the control until 6weeks after heading (September 13th) (Fig.2.5).

Arao et al. (2018) reported that the daily mean temperature during 2–4 weeks post-heading was significantly correlated with the iAs concentrations in the rice grains, indicating that this period (2–4 weeks post-heading) is important in the uptake and accumulation of As in rice grains. The accumulation of As in brown rice has been shown to exhibit a low rate of translocation from other organs, and a very high percentage of As is absorbed during the ripening period (Tyrovola and Nikolaidis, 2009; Bonte et al., 2013). We thus suggest that in the present study, the rice plants grown in the high temperature was more likely to absorb As due to the greater amounts of As released from the soil.

4.2. The decrease in the rice yield due to high temperature and yield components

A high temperature after heading in rice seems to reduce the rice yield via a reduction of the ripening percentage as well as an increase of the imperfect or empty grain numbers per panicle (Table 2.3). In the study conducted at an International Rice Research Institute farm, Peng et al. (2004) demonstrated that the mean air temperature and the minimum air temperature decreased the grain yield by 15% and 10%, respectively with each 1°C increase in the air temperature during the rice-growing season. Kim et al. (2011) reported that compared to 21 °C, at 27 °C the grain filling speed was increased and the grain duration was shortened, along with a significantly reduced grain weight due to the influence of the ripening ratio. Due to the combined effect of increased temperature and soil As, the yield decreased by 42%, suggesting a major impact on rice yield and grain quality (Muehe et al., 2019). Similar results were observed in our experiment; i.e., high temperature caused a decrease in yield through a decrease in the ripening rate.

We noted an inconsistent effect of high temperature on the thousand-grain weight. The thousand-grain weight was tended to be increased in the high-temperature treatment. We speculate that the higher thousand-grain weight in the high-temperature treatment compared to the control could be a result of the decrease in the number of effective grains for ripening due to the influence of high temperature. In this case, the amount of carbohydrate would be preferentially accumulated in the remaining grains, resulting in an increase of the thousand-grain weight while the total grain yield was decreased.

4.3. The effect of high temperature on the As concentration in the brown rice

In the present study, the meaningful effect of temperature during the ripening period on the concentrations of iAs in the brown rice was detected ($p = 0.065$), the iAs concentration was higher in the high-temperature treatment than the control (Table 2.4). This result suggests that the temperature during the ripening period is an important factor in increasing the As concentration in brown rice. We suspect that these results are related to the increase in the As concentration in the soil solution due to the increase of As from the soil, which was due to the increase in soil temperature. Neumann et al. (2017) observed that the soil As was solubilized as the soil temperature increased, and the concentration of As in the rice straw and husk also increased when the rice was grown in a root box (30 cm × 20 cm × 2.5 cm) with the air temperature maintained at the same level and only the soil temperature changing. However, Neumann et al. (2017) also showed that the concentration of As in the rice grains did not differ among the treatments. It is thus conceivable that the cause of the increase in the As concentration in rice grains is not only an increase in the supply of As from the soil due to an increase in soil temperature but also the influence of the translocation to rice grains caused by the increase in air temperature.

It was reported that the translocation of As absorbed into the above-ground part of rice is sequestered in the vacuole of the cells (which is associated with the phloem of the node), and that the translocation of As into brown rice is suppressed (Yamaji and Ma, 2014; Chen et al., 2015). It was also clarified that As is detoxified by binding to glutathione (GSH) and phytochelatin (PC) and accumulates in vacuoles (Suriyagoda et al., 2018), and that the translocation of iAs into brown rice can be achieved with the use of a sieve tube (Carey et al., 2011). Therefore, in order to elucidate the mechanisms underlying the increase in the As concentration in rice grains due to high temperature, it is necessary to determine the effect of high temperature on the mechanisms of As accumulation and defense in rice plants.

4.4. The risk of human As intake from rice cultivated under high temperature

In this study, uncontaminated soil with relatively low available As concentration (1 mg kg^{-1}) was tested. Since the average available As concentration in Japanese paddy soil is 1-2 mg kg^{-1} (Arao et al., 2011), that used in this study was slightly lower than the average. In general, rice cultivated on contaminated soil or soil with extremely high As concentration is very unlikely to reach the market. Therefore, it seemed to be better to conduct experiments using soil where the As concentration of the soil is not so high and suitable for the evaluation of food production and distribution and food safety.

According to a survey of rice As concentrations by the Ministry of Agriculture, Forestry and Fisheries of Japan, approximately 5 % of brown rice cultivated in Japan collected in 2012 contained iAs at concentrations higher than 0.35 mg kg^{-1}, and the average concentration (n = 600) of iAs was 0.21 mg kg^{-1} for brown rice (MAFF, 2014). It was higher than that in brown rice collected from 2004 to 2006 (n = 600) by 0.15 mg kg^{-1}. Although the cause of the higher iAs concentration in 2012 is unknown, differences in the weather conditions in each year could have contributed to the differing iAs concentrations in rice. In the present study, the concentration of brown rice in the control was 0.219 mg kg^{-1} (Table 2.4), which was similar to the results of the 2012 Ministry of Agriculture and Fisheries survey. We observed that the concentration of iAs in the brown rice was significantly increased when the ripening period was under a high-temperature condition (Table 2.4). Indeed, the absolute value of the difference is small, but even in the case of using such low As soil, the fact that high temperature significantly increases the brown rice iAs concentration is meaningful to make recommendations on food safety. Therefore, it is necessary to discuss future risks based on the results of experiments on soils with relatively high arsenic concentrations.

Muehe et al. (2019) reported important information on the relationship between elevated temperatures and soil As content, and warned of possible future rice cultivation at elevated temperatures. That is, they conducted rice cultivation experiments at two temperatures, 33 °C (the average daily temperature during the rice cultivation period from May to September in California) and 38 °C, which is 5 °C higher than that. When cultivated at 38 °C, the soil As increased, thus increasing the concentration of rice grain As to 1004 ± 17.9 μg kg^{-1} grains, of which 400 μg kg^{-1} showed to be iAs. They noted that an increase in the As concentration in rice grains with the increase in temperature could seriously affect humans.

In many parts of the world, rice is considered one of the potential routes of As exposure (Mondal and Polya, 2008; Chatterjee et al., 2010). In an evaluation of the As risk for humans, the weekly intake of iAs for a 70-kg adult was lower than the provisional tolerable weekly intake (PTWI) value, but children <3 years old were the most exposed to iAs (Pasias et al., 2013). In the present study, we observed a decrease in the grain yield and an increase in iAs concentration in the brown rice under the high-temperature conditions during the ripening period. In other words, if rice is produced under the trend of high temperatures in the near future, the risk of human As intake will be increased. Further investigations of agronomic methods are required in field conditions to determine all of the precise effects of increased temperatures.

Chapter 3

The Increase in the Arsenic Concentration in Brown Rice Due to High Temperature during the Ripening Period and Its Reduction by Silicate Material Treatment

This chapter presents results of the assessment of daily air and soil temperature, arsenic in biomass production, and brown rice yield. It summarizes results on the presence of high temperature effect on As accumulation in brown rice grain one week after heading during the ripening period. It also evaluated the reduction in brown rice yield mitigated by the application of calcium Silicate materials even in different high temperature conditions with lowland As uncontaminated paddy soil at TGCs. Its also evaluated the results of high temperature effect during the ripening period and the Si application on the As concentration and As speciation in brown rice.

Abstract

Rice grown in Wagner pots (1/5000a) was placed in three TGCs (each TGC was set at four temperature levels: ambient, mildly-high temperature, moderately-high temperature, and super-high temperature) from one week after heading until harvest. In the TGCs, a range of mean air temperatures was observed in the range of 2 ◦C above the ambient temperature. 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 the application of calcium silicate. 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 was applied, the concentration of As in brown rice was significantly lower at all temperature ranges, and its application was effective in reducing the arsenic concentration even at high temperatures. These results suggest that the application of silicate material may help mitigate the decrease in yield and the increasing As concentration in brown rice even under high-temperature conditions.

1.Introduction

The Fifth Assessment Report of the Intergovernmental Panel on Climate Change (IPCC, 2017) states that "the observed global warming since the mid-20th century has a high probability of being anthropogenic more than 95%". This trend of high temperatures is sure to continue, and there are global concerns about the impact of high temperatures on agricultural products. In paddy rice cultivation, in addition to the problems caused by climate change, high-temperature growth disturbances that significantly reduce the yield and quality of rice are frequent and problematic (Morita, 2008). Peng et al. (2008) directly proved that the increase in nighttime temperature due to global warming reduces the rice yield. Morita, and Nakano (2011) also reported that at temperatures above the optimum growing temperature, dry matter production is inhibited due to small grain size, lower maturity, lower grain quality, sterility, and consequently lower grain yield. Against this background indicating that changes in temperature due to global warming may lead to a decrease in rice yield and quality, Arao et al. (2018) analyzed the concentration of arsenic (As) in rice grains produced between 1995 and 2014, and their results showed a significant correlation between the concentration of As in rice and the daily mean temperature (DMT) between two and four weeks after the emergence of the rice in the year in which it was produced, suggesting that the higher the temperature during the rice ripening period, the higher the concentration of inorganic As (iAs) in the rice grains. The Codex Alimentarius Commission (2016) established upper limits for the permissible concentration of iAs in milled and brown rice as 0.20 mg kg^{-1} and 0.35 mg kg^{-1}, respectively. Arsenic is widely distributed in nature, and it is contained in the soil at an average of 11 mg kg^{-1} (Kabata-Pendias, 2000). Ferns, known as arsenic hyperaccumulators, show concentrations of up to 2 percent by dry weight (Ma et al., 2001). However, it is known that most of the field crops do not generally show concentrations above 1 mg kg^{-1} (Allaway, 1968). In contrast, stems and leaves of paddy rice can usually reach about 5 mg

kg^{-1} even when grown in unpolluted soil (Feng et al., 2017). The difference in the As concentration between many field crops and paddy rice is greatly influenced by the form of As species in their cultivated soil. Since the upland soil is oxidative, As exists as pentavalent arsenate and is strongly bound to iron and aluminum in the soil so that it is not eluted into the soil solution (Takahashi et al., 2004). Therefore, many field crops do not readily absorb As. On the other hand, since paddy fields are in a reducing condition, As is easily reduced to highly soluble trivalent arsenite and is easily eluted into the soil solution so that paddy rice absorbs As easily (Takahashi et al., 2004). When the As concentration was compared by parts, the highest concentration of As was found in the roots, followed by the stems and leaves, and the rice grain was the lowest (Feng et al., 2017). For example, the concentration of As in rice grains grown in soil containing a high concentration of arsenic is unlikely to exceed 1 mg kg^{-1} (Zavala and Duxbury, 2008). Zavala and Duxbury (2008) obtained rice produced or distributed around the world, and the variation in the concentration of As was investigated. They found a considerable variation of 0.005 to 0.710 mg kg^{-1} in total As concentration. Furthermore, they estimated that the normal concentration of As in rice in the world is in the range of 0.08 to 0.20 mg kg^{-1} by considering the literature values. Rice is a staple food for about half of the world’s population, but it is also the most common source of iAs, a class 1 carcinogen (Meharg et al., 2009). It is essential to do research to clarify the mechanism of As accumulation in brown rice. It is known that there are various defense mechanisms when As is absorbed from the soil (Ashraf et al., 2020; Verma et al., 2020) and is transferred to brown rice (Yamaji and Ma, 2014). In recent years, proteomic analysis and genetic analysis studies have been conducted on the As-trapping function in nodes (Song et al., 2014). As mentioned above, there is a concern that the As concentration in rice grains will increase due to the global temperature rise, and it will therefore be necessary to reduce the As concentration even in rice grown in uncontaminated soil. A temperature gradient chamber (TGC) was designed to provide the appropriate technology to develop an experimental facility to study the response of rice to temperature stress and changes in the global environment (Okada et al., 1995). The TGC is a highly effective system for studying the effects of temperature on crop cultivation because it allows for high-temperature treatment linked to the actual temperature. In paddy rice cultivation, it is necessary to investigate the effect of temperature on the As accumulation and speciation of rice grains in order to understand the effect of temperature increases on the rice yield and the As concentration. The present experiments were conducted on uncontaminated soil with relatively low As concentrations. Because rice grown in contaminated soils or soils with extremely high concentrations of As is very unlikely to be marketed, we thought it would be better for the evaluation of the actual production and distribution of rice and the safety of the food to conduct the experiments in average paddy soils where the As concentrations are not so high. Many countermeasures to reduce the As concentration in rice have been reported, such as breeding varieties with low As absorption, water-saving cultivation, and the application of soil amendments to reduce the As absorption Matsumoto et al., 2015a; Li et al., 2009a; Zhao et al., 2010a). However, the breeding of low-absorption varieties requires an extended period. Water-saving cultivation is problematic in terms of reduced yield and quality as well as an accelerated absorption of cadmium. In contrast, the application of soil amendment is a practical measure for farmers because it is general manure management. We have shown that the application of silicic materials reduces the As concentration in brown rice (Matsumoto et al., 2015a, b; Matsumoto et al., 2016), but it has not been established whether the silicic acid application can reduce the concentration of brown rice As even at high temperatures. We conducted the present study to clarify the relationship between the temperature and the concentration of As in brown rice by setting the temperature level after emergence with TGCs, and we investigated whether the silicic acid application is effective in reducing the concentration of As in brown rice even under the increased temperature.

2. Materials and Methods

2.1. Soil Preparation, Si Material Treatment and Rice Cultivation

For the rice cultivation, we filled Wagner pots (1/5000a, Fujiwara Scientific, Tokyo, Japan) with 3 kg of As-uncontaminated gray lowland paddy soil collected from the plowed layer of a paddy field at Matsue, Shimane, Japan. In every pot we mixed 2.8 g of a compound fertilizer that contained N 14%, P_2O_5 14%, and K_2O 14%, applied as a basal fertilizer before the seedlings were transplanted. Calcium silicate slag (SiO_2 , 30%), which is the most widely used silica (Si) material in Japan, was also applied at 7.5 g per pot as a Si application treatment. The application rate of calcium silicate slag is equivalent to 1.5 times the recommended standard in Japan. No calcium silicate slag was applied to the control pots. The tested soil contained 1.6% total C, 0.14% total N, 1.2 mg kg^{-1} of available As (1 M HCl extractable form), and 44.2 mg kg^{-1} of available SiO_2. This soil had a pH of 5.5. This soil tested was not unpolluted with relatively low As concentration. A popular Japanese cultivar (*Oryza sativa* L. cv. Koshihikari) was selected for rice cultivation. Hastening of germination was conducted in an incubator at 32 ◦C under dark conditions for 24 h. Germinated seeds were sown in paper pots (comprised of 578 1.5-cm^2, 3-cm-deep blocks). Rice seedlings that were approx. 25 days old were transplanted to the Wagner pots on 31 May, 2018. In the center of each pot, three seedlings were transplanted, and flooding conditions were maintained from the transplanting to harvesting. At 48 days after the seedlings were transplanted, a compound liquid fertilizer containing 0.21% N as a form of $(NH_4)_2SO_4$ was applied to each pot with 0.5 g of N. The plants were grown outdoors for 71 days before the start of the high-temperature treatment.

2.2. High-Temperature Treatment during the Ripening Period

Three 15-m-long TGCs (Figure 3.1) were used to investigate the As accumulation in rice grains under high-temperature conditions. For the high-temperature treatments, the TGCs were set up in the paddy field at Shimane University, Matsue, Japan (35◦290 N, 133◦040 E, 4 m asl). To detect the temperature variation, we applied four temperature regimens, in order, from the entrance of the TGC to deep inside the TGC: ambient, mildly-high, moderately-high, and super-high-temperature treatments. The boundaries of each treatment section were separated by a transparent nylon curtain with vertical slits. A high-quality fan was attached on the back end of each TGC and continuously provided a gentle airflow from the entrance to deep inside the TGC to create more warm heat. This set-up provided an effective way to understand and measure the temperature variation from each of the four sections of each TGC. The temperature in the mildly-high plot is close to the outside temperature (ambient plot) because the airflow passes first through the entrance, and the temperature gradually increases toward the far end of the TGC (Figure 3.2). The daytime air temperature was set not to exceed 40 ◦C by adjusting the fan's exhaust speed. The air temperatures and soil temperatures were recorded until harvesting.

The heading date when 50% of the total heading occurred was August 3rd. Seven days after the heading date (August 10th), six pots (three control pots and three Si-application pots) were transferred to each of the four temperature treatments in each of the three TGCs. A total of 72 pots (6×4×3) was thus used in this study. These pots were grown in the TGCs until harvesting (September 19th).

2.3. Plant Sampling

We harvested the rice plants at maturity (19 September) from each pot for the evaluation of the As concentration in rice plant tissues. The harvested plant samples were air-dried in a greenhouse for 7 days. The air-dried samples were divided into straw and panicles. Husks and unfilled grains were removed for only the analyses of the As and speciation concentrations in brown rice grains. The panicles were divided into rachis and grains. Unfilled grains were removed by ammonium sulfate solution, and the grains were counted by a machine (IC-1, Aidex Co., Nagoya, Japan). Filled grains were de-husked by a machine (FC2K, Otake Agricultural Machinery Co., Aichi, Japan).

2.4. Chemical Analyses of the As and Speciation in the Plant Tissues

Five mL of 5:1 (v/v) HNO3/H2O2 was added to each powdered sample of brown rice (0.5 g), and the samples were wet-digested in a microwave oven (MLS 1200, Milestone, Bergamo, Italy). The concentrations of As in the degraded samples was determined by inductively coupled plasma mass spectrometry (ICP-MS) (ELAN DRC-e, PerkinElmer Sciex, Shelton, CT, USA). We determined the As speciation in the rice grains by using the method of Matsumoto et al. (2016). Powdered brown rice (0.5 g) was mixed with 2 mL of HNO_3 (0.15 M) in a 10-mL capped high-density polyethylene centrifuge tube, and the mixture was heated in an aluminum heating block at 80 °C for 2 h. The obtained extract was diluted to 10 mL with water and passed through a 0.45-μm filter before analysis. The As speciation of diluted solution samples was determined by a high-performance liquid chromatography (HPLC)/ICP-MS system. A Super IC-Anion column (5 μm ID, 4.6 mm ID, 150 mm ID) and a guard column (Tosoh, Tokyo) equipped with an isocratic mobile phase system consisting of 10 mM ammonium acetate and an HPLC system (PU 712i, GL Sciences, Tokyo, Japan) were used. The injection volume and mobile-phase flow were set at 10 and 800 μL min^{-1}, respectively. Arsenic concentration was determined using the ELAN DRC-e ICP-MS system.

Total inorganic As (iAs) is expressed as the sum of arsenite and arsenate. The accuracy of the analysis was certified by the reference material (CRM) (rice flour, NMIJ CRM 7503-a: arsenite 0.0711±0.0029 mg kg^{-1}, arsenite 0.0130±0.0009 mg kg^{-1}, dimethyl arsenate (DMA) 0.0133±0.0009 mg kg^{-1}; National Institute of Advanced Industrial Science and Technology (NIJM), Tsukuba, Japan).

2.5. Statistical Analyses

The representative values of growth and As concentration in each temperature treatment in each TGC were taken as the average of three pots. The effects of temperature on the growth and As concentrations in brown rice were validated by an analysis of covariance (ANCOVA) with air or soil temperature as a covariate. We also tested the significance of the control and Si application when the ANCOVA was significant.

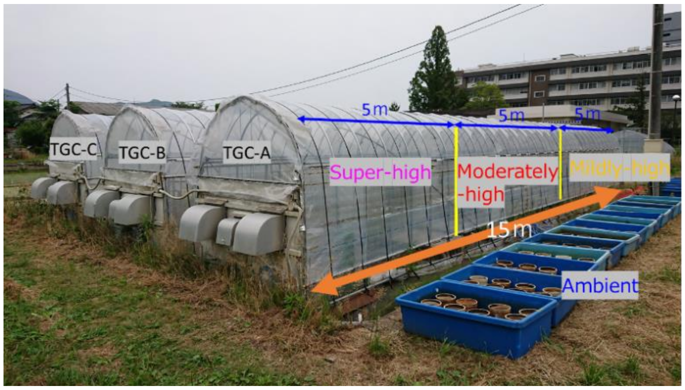


Figure 3.1. Three temperature gradient chambers (TGCs) used for high-temperature treatment during the ripening period.

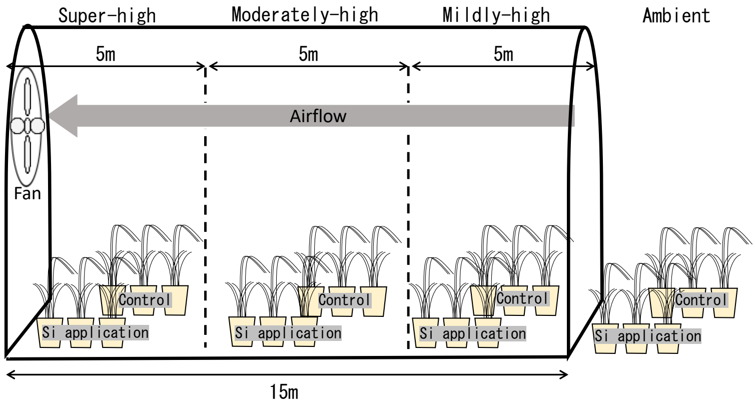


Figure 3.2. Schematic inside of TGC.

3. Results

3.1. Temperature Differences between the Ambient, Mildly-High, Moderately-High, and Super-High Treatments in the TGCs during the Ripening Period

The daily air temperature differences among the four temperature treatments for the T_{mean}, T_{max}, and T_{min} in the TGCs during the ripening period are shown in Figure 3.3. According to the daily temperature data, the differences in the average daily air temperatures among the four treatments (i.e., ambient, mildly-high, moderately-high, and super-high temperatures) were 1.1 °C–2.0 °C (T_{mean}), 3.6 °C–4.0 °C (T_{max}), and 0.10 °C–0.9 °C (T_{min}), respectively. As summarized in Table 3.1, the average daily mean air temperature during the ripening period was 25.9 °C in the ambient sections of the TGCs, 27.0 °C in the mildly-high sections, 27.9 °C in the moderately-high sections, and 27.8 °C in the super-high temperature sections. Therefore, the greatest difference between the average daily mean temperatures observed in the daily temperature data during the ripening period was 2.0 °C.

The average daily maximum air temperature was 30.6 °C in the ambient-temperature sections, 33.6 °C in the mildly-high, 34.6 °C in the moderately-high, and 34.5 °C in the super-high; as a result, the difference in the average daily maximum air temperatures among the four temperature treatments during the ripening period was 3.0 °C.

In contrast, the data in Table 3.1 show that the average daily air minimum temperature was 22.7 °C in the ambient, 22.6 °C in the mildly-high, 23.7 °C in the moderately-high, and 23.6 °C in the super-high sections during the ripening period. From the daily minimum temperature data, the difference in the average daily air minimum temperature during the ripening period was 1.1 °C. Based on the daily minimum temperature data, there were clear temperature differences between the four high-temperature treatments. We thus observed an effective and meaningful air temperature difference between the ambient-temperature sections of the TGCs and the three high-temperature treatments.

The daily soil temperature differences are shown in Figure 3.4 for the four temperature sections in the TGCs for the T_{mean}, T_{max}, and T_{min}. The variations in the daily soil temperature differences among the ambient, mildly-high, moderately-high, and super-high treatments were 0.2 ◦C–3.3 ◦C (T_{mean}), 0.5 ◦C–2.6 ◦C (T_{max}), and 0.5 ◦C–1.7 ◦C (T_{min}). The average daily soil temperature differences for T_{mean} and T_{max} between the ambient and high-temperature treatments differed slightly among the three TGCs. Generally, the range of temperature differences for T_{min} was smaller than that for T_{max} during the ripening period.

The average daily mean soil temperature during the ripening period was 25.7 ◦C in the ambient, 26.9 ◦C in the mildly-high, 27.5 ◦C in the moderately-high, and 28.0 ◦C in the super-high sections (Table 3.1); thus the difference in the soil average daily mean temperatures among the four treatments was high, at 2.3 ◦C. However, our evaluation showed that the average daily soil maximum temperature during the ripening period was 27.9 ◦C in the ambient, 29.5 ◦C in the mildly-high, 30.0 ◦C in the moderately-high, and 30.5◦C in the super-high sections of the TGCs. We had expected that the daily soil maximum temperature difference would be high. The average daily soil minimum temperature during the ripening period was 24.3 ◦C in the ambient, 24.9 ◦C in the mildly-high, 25.5 ◦C in the moderately-high, and 26.0 ◦C in the super-high sections. The difference in the average daily minimum temperatures during the ripening period was thus 1.7 ◦C. Based on these soil temperatures data, we detected an effective soil temperature difference among the temperature treatments.

3.2. Effects of Temperature during Ripening Period and Si Application on the Biomass Production and Brown Rice Yield

The data of the dry matter production (straw and panicle) and brown rice yield are shown in Supplementary Table 3S1. These data were analyzed by ANCOVA with the average air or soil temperature after heading as a covariate and Si application as a fixed factor (Table 3.2). No significance of regression was observed for the straw weight. We thus consider that the straw weight was not affected by the increases in the air and soil temperatures after heading. In contrast, the significance of regression was recognized for the panicle weight and brown rice yield; that is, it was confirmed that the panicle weight and brown rice yield decreased significantly as the air and soil temperatures increased during the ripening period. The ANCOVA revealed a significant effect of the Si application. The Si application significantly increased the dry matter production (straw and panicle) and the brown rice yield in all of the temperature ranges. Thus, the application of silicate effectively increased the dry matter production and brown rice yield even in high-temperature conditions during the ripening period.

3.3. Effects of Temperature during the Ripening Period and the Si Application on the As Concentration and As Speciation in Brown Rice

The results of our analyses of As in brown rice are summarized in Supplementary Table 3S2, and the relationships between the As concentration and the temperature and Si application are illustrated in Figure 3.5. The proportions of the concentration of the sum of As species to that of total As in the brown rice ranged from 90% to 97% (mean: 94%), indicating that the accuracy of the speciation analysis was reasonable (Table 3S2). The average proportion of the concentration of iAs to total As was 73.7%, and as reported (Matsumoto et al., 2016), iAs was the dominant form of As present in the brown rice, and the degree of the changes with temperature treatment and Si application was small. The average proportion of DMA to total As was approx. 20%, and the variation between treatments was small.

The concentrations of iAs and DMA increased significantly with the increasing air and soil temperatures during the ripening period in both the control and Si application (Figure 3.5). The monomethyl arsonate (MMA) concentration was extremely low in all treatments, and its relationships with the air and soil temperatures were not clear. As mentioned above, since the dominant forms of As in brown rice were iAs and DMA, similarly, the concentration of total As and the sum of As species also increased significantly with the increasing temperatures. The concentration of As (except MMA) in the brown rice tended to be lower in the Si application than in the control in any temperature range. Therefore, for the As species that showed a linear relationship with these temperatures, we performed an ANCOVA using the soil temperature and air temperature as covariates, and we analyzed the As reduction effect of the Si application under elevated temperature. The results of the ANCOVA revealed significant linear relationships between the concentrations of iAs, DMA, the sum of As species, and total As with the average air and soil temperatures during the ripening period (Table 3.3). The effect of the Si application on the concentration of iAs was nonsignificant when the significance level of 5% was used but was significantly reduced at the significance level of 10%. The concentrations of DMA, the sum of As species, and the total As were significantly reduced by the Si application at a significance level of The concentrations of DMA, the sum of As species, and the total As were significantly reduced by the Si application at a significance level of <0.1%. These results confirmed that the increase in air and soil temperatures during the ripening period significantly increased the As concentration in brown rice, but the Si application was effective in reducing the As concentration in brown rice compared to the control at all of the temperatures used herein.

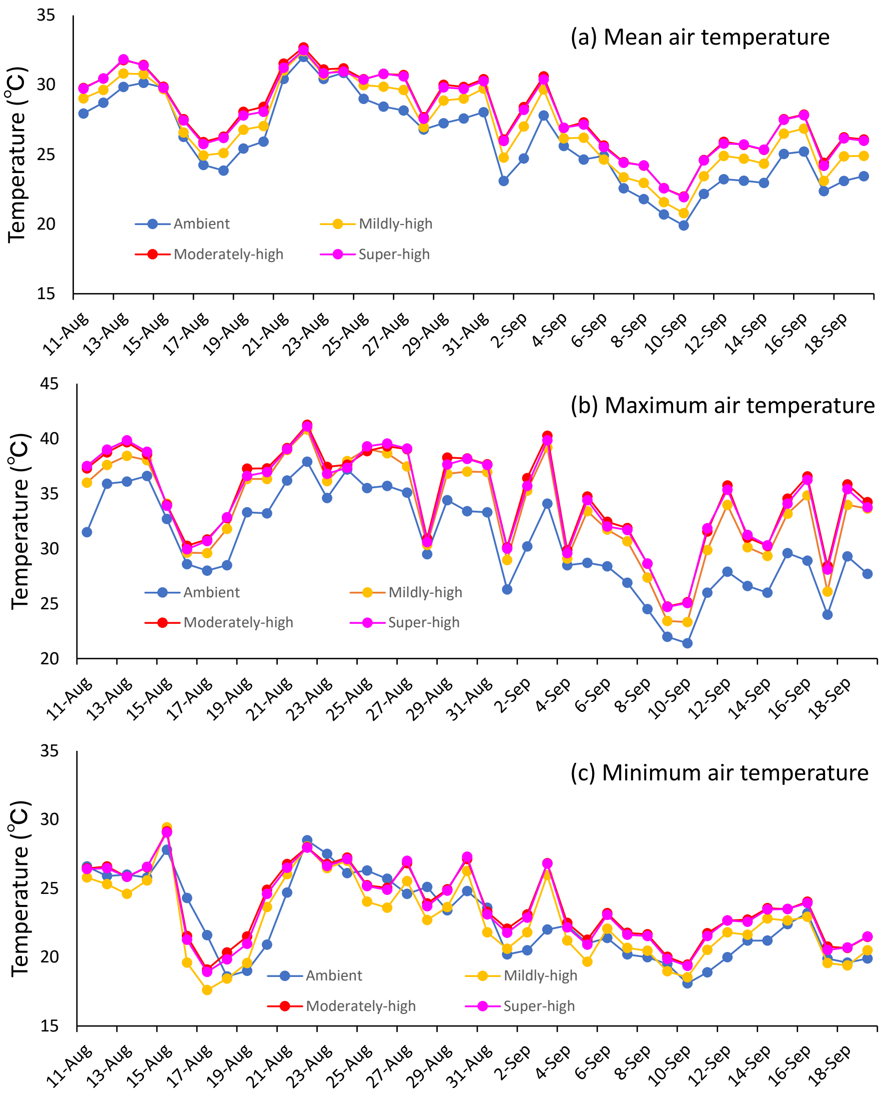


Figure 3.3. Changes in average daily mean air temperature (a), daily maximum air temperature (b), and daily minimum air temperature (c) during the ripening period.

Table 3.1. Average air and soil temperature during ripening period.

Temperature	Air temperature (℃)			Soil temperature (℃)		
	Mean	Maximum	Minimum	Mean	Maximum	Minimum
Ambient	25.9	30.6	22.7	25.7	27.9	24.3
Mildly-high	27.0	33.6	22.6	26.9	29.5	24.9
Moderately-high	27.9	34.6	23.7	27.5	30.0	25.5
Super-high	27.8	34.5	23.6	28.0	30.5	26.0

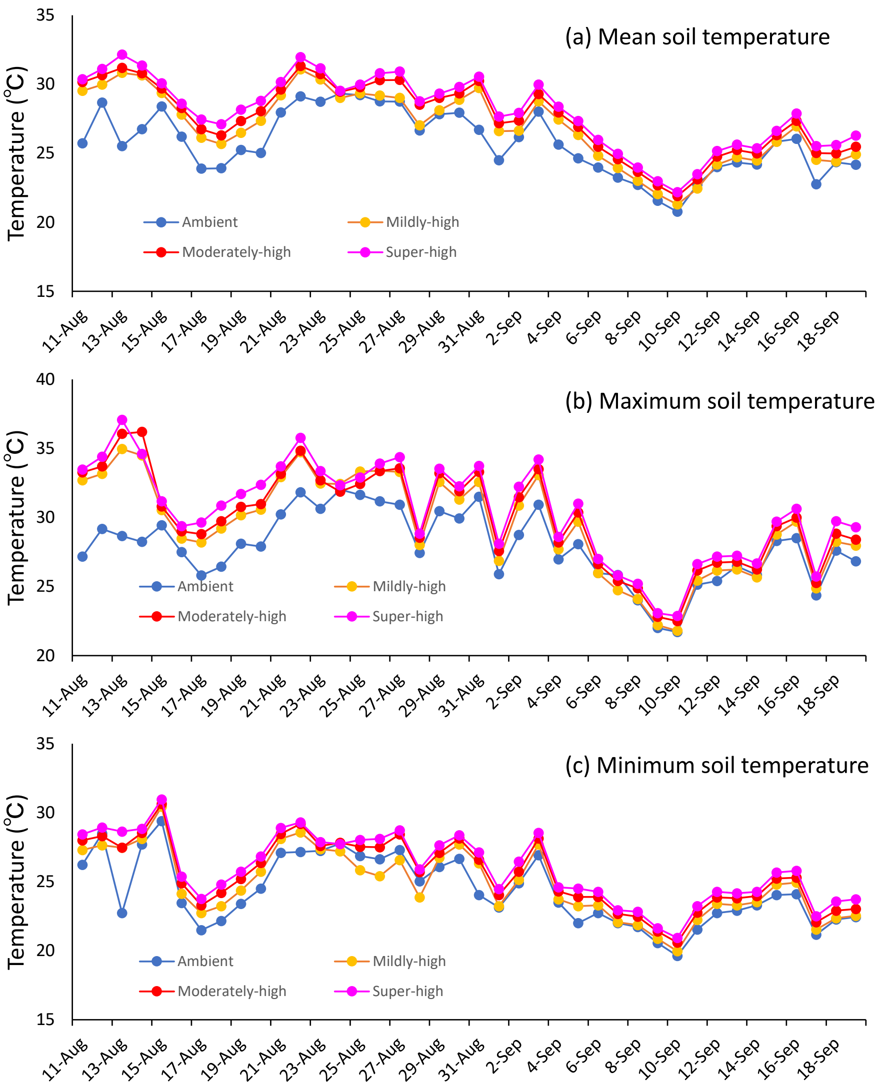
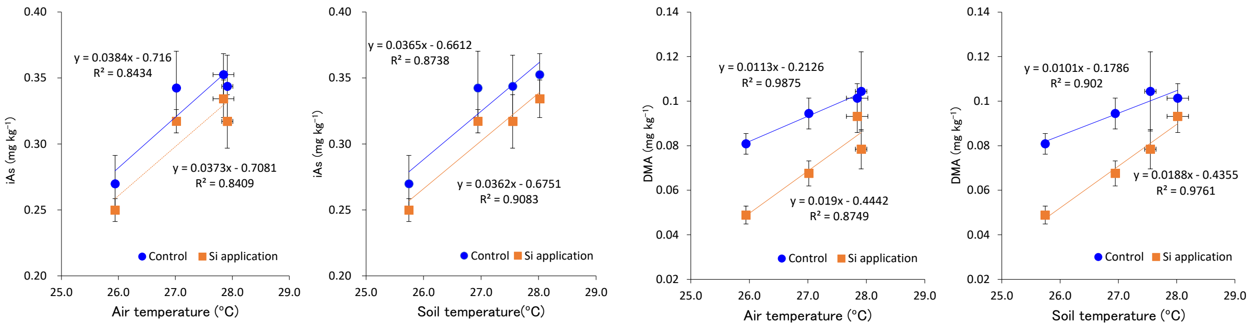


Figure 3.4. Changes in average daily mean soil temperature (a), daily maximum soil temperature (b), and daily minimum soil temperature (c) during the ripening period.

Table 3.2. Effect of the temperature and Si application on the dry matter production and brown rice yield.

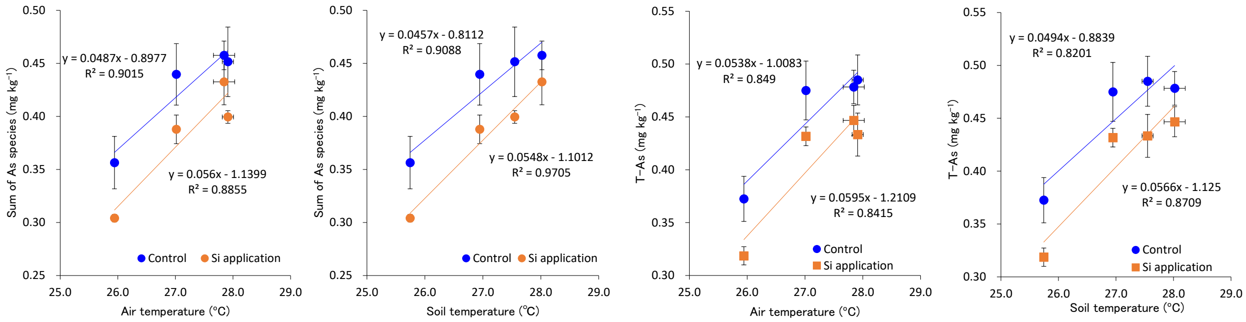
Main Factor	Treatment	Dry matter production		Brown rice yield (g pot^{-1})
		Straw (g pot^{-1})	Panicle (g pot^{-1})	
Temperature	Ambient	18.5 ± 1.2	22.9 ± 1.2	18.5 ± 1.0
	Mildly-high	18.7 ± 1.6	16.7 ± 1.3	12.4 ± 0.9
	Moderately-high	20.4 ± 2.6	15.6 ± 2.0	11.3 ± 1.5
	Super-high	20.2 ± 2.3	16.4 ± 2.3	11.9 ± 1.7
Si application	Control	15.8 ± 0.6	14.7 ± 1.1	11.2 ± 1.0
	Calcium silicate slug	23.1 ± 1.0	21.1 ± 1.1	15.9 ± 1.0
Ancova Significance of regression	Mean air temperature	$p = 0.125$	$p < 0.001$	$p < 0.001$
	Mean soil temperature	$p = 0.192$	$p < 0.001$	$p < 0.001$
Si application	Mean air temperature	$p < 0.001$	$p < 0.001$	$p < 0.001$
	Mean soil temperature	$p < 0.001$	$p < 0.001$	$p < 0.001$

Mean values ± standard errors (Temperature, n = 6; Si application, n = 12).



(a) Inorganic As

(b) DMA



(c) Sum of As species

(d) Total As

Figure 3.5. Relationships between air and soil temperature during the ripening period and the concentrations of arsenic species in brown rice.

Table 3.3. Effects of the temperature treatment during the ripening period and Si application on the As concentrations in brown rice.

Main Factor	Treatment	Inorganic As (mg kg^{-1})	DMA (mg kg^{-1})	MMA (mg kg^{-1})	Sum of As species (mg kg^{-1})	Total As (mg kg^{-1})
Temperature	Ambient	0.260 ± 0.011	0.065 ± 0.008	0.005 ± 0.000	0.330 ± 0.016	0.346 ± 0.016
	Mildly-high	0.330 ± 0.014	0.081 ± 0.007	0.003 ± 0.000	0.414 ± 0.018	0.453 ± 0.016
	Moderately-high	0.330 ± 0.009	0.091 ± 0.011	0.004 ± 0.000	0.425 ± 0.019	0.459 ± 0.018
	Super-high	0.343 ± 0.011	0.097 ± 0.005	0.004 ± 0.001	0.445 ± 0.013	0.463 ± 0.012
Si application	Control	0.327 ± 0.013	0.095 ± 0.005	0.004 ± 0.000	0.426 ± 0.017	0.453 ± 0.017
	Calcium silicate slug	0.305 ± 0.011	0.072 ± 0.006	0.004 ± 0.000	0.381 ± 0.015	0.408 ± 0.017
Ancova Significance of regression	Mean air temperature	$p < 0.001$	$p < 0.001$	$p = 0.071$	$p < 0.001$	$p < 0.001$
	Mean soil temperature	$p < 0.001$	$p < 0.001$	$p = 0.150$	$p < 0.001$	$p < 0.001$
Si application	Mean air temperature	$p = 0.067$	$p < 0.001$	$p = 0.458$	$p = 0.005$	$p = 0.008$
	Mean soil temperature	$p = 0.061$	$p < 0.001$	$p = 0.471$	$p = 0.004$	$p = 0.007$

Mean values ± standard errors (Temperature, n = 6; Si application, n = 12).

Table 3.S1. Dry matter production and brown rice yield in the present study.

Temperature treatment	Si application	Dry matter production Straw (g pot^{-1})	Dry matter production Panicle (g pot^{-1})	Brown rice yeild (g pot^{-1})
Ambient	Control	16.7 ± 3.0	20.7 ± 0.2	19.7 ± 0.2
Mild high	Control	15.5 ± 2.0	14.4 ± 2.4	12.5 ± 2.1
High	Control	15.6 ± 2.4	12.3 ± 0.9	10.3 ± 0.7
Super high	Control	15.5 ± 1.1	11.7 ± 0.1	9.9 ± 0.2
Ambient	Apply	20.3 ± 1.7	25.1 ± 2.7	23.9 ± 2.5
Mild high	Apply	21.9 ± 0.9	19.1 ± 1.2	16.6 ± 1.2
High	Apply	25.2 ± 5.1	18.9 ± 5.3	16.4 ± 4.6
Super high	Apply	25.0 ± 2.8	21.1 ± 3.4	18.2 ± 3.2

Table 3.S2. Concentrations of As species in brown rice in the present study.

Temperature treatment	Si application	Inorganic As (mg kg^{-1})	DMA (mg kg^{-1})	MMA (mg kg^{-1})	Sum of As species (mg kg^{-1})	Total As (mg kg^{-1})
Ambient	Control	0.270 ± 0.037 (73)	0.081 ± 0.008 (22)	0.006 ± 0.001 (1.6)	0.356 ± 0.043 (97)	0.373 ± 0.037
Mild high	Control	0.342 ± 0.048 (72)	0.094 ± 0.012 (20)	0.003 ± 0.000 (0.6)	0.440 ± 0.050 (93)	0.475 ± 0.048
High	Control	0.344 ± 0.041 (71)	0.104 ± 0.031 (21)	0.003 ± 0.001 (0.7)	0.451 ± 0.057 (93)	0.485 ± 0.041
Super high	Control	0.353 ± 0.028 (74)	0.101 ± 0.011 (21)	0.004 ± 0.002 (0.8)	0.458 ± 0.023 (96)	0.478 ± 0.028
Ambient	Calsium silicate slug	0.250 ± 0.015 (78)	0.049 ± 0.007 (15)	0.005 ± 0.001 (1.7)	0.304 ± 0.006 (96)	0.319 ± 0.015
Mild high	Calsium silicate slug	0.317 ± 0.015 (73)	0.068 ± 0.010 (16)	0.003 ± 0.001 (0.7)	0.388 ± 0.023 (90)	0.432 ± 0.015
High	Calsium silicate slug	0.317 ± 0.035 (73)	0.078 ± 0.015 (18)	0.004 ± 0.001 (0.9)	0.399 ± 0.010 (93)	0.433 ± 0.035
Super high	Calsium silicate slug	0.334 ± 0.025 (75)	0.093 ± 0.012 (21)	0.005 ± 0.002 (1.2)	0.433 ± 0.037 (97)	0.447 ± 0.025

Numbers in parentheses indicate the percentage of each As species to total As.

Mean values ± standard errors (n = 3).

4. Discussion

The significant effects of high global temperature on global food production are a severe problem that needs urgent action. As many researchers predicted, increased temperatures can reduce the rice production yield. Tao et al. (2008) used the 30-year period of 1961–1990 climatic data in their efforts toward the development of effective techniques to combat the effects of global warming on rice production. They reported that at 1 ◦C, 2 ◦C, and 3 ◦C increases in the mean temperature, the decreases in the rice yield were 6.1%–18.6%, 13.5%–31.9%, and 23.6%–40.2%, respectively (Tao et al., 2008). In our present investigation, the average temperature observed during the ripening period in the three high-temperature treatments in the TGCs was up to 2 ◦C higher than the ambient treatment's temperature (25.9 ◦C). Similarly, a range of 2.3 ◦C higher mean soil temperature was observed compared to 25.7 ◦C for the ambient treatment. In this situation, a significant negative regression equation was obtained for the brown rice yield with increasing air and soil temperatures, and the brown rice yields in the moderately-high temperature treatment and super-high temperature treatment were approx. 39% lower than the ambient treatment.

The rate of photosynthesis decreases when the temperature increases above the optimum value for plant growth (Van Kiet, 2016), and it has thus been shown that a reduced ripening period leads to an inadequate accumulation of carbohydrates and to a decrease in yield due to increases in sterility and immature grains (Horie et al., 2000; Jin et al., 1995; Morita et al., 2016; Agostinho et al., 2017; Matoh, 1991). In the present study, the yields were significantly higher in the Si application compared to the control. Even in the Si application, the yield was significantly reduced by high temperature, but the yield reduction was lessened compared to the control. In other words, our results demonstrated that Si application would be an effective agronomic management technique to minimize yield losses under high-temperature conditions.

Agostinho et al. (2017) suggested that the application of different Si sources increased not only the biomass Si content but also the Si content at the tillering, booting, and flowering stages by 12%, 10%, and 23% respectively, and that the increase in Si content increased grain yield by 4.46%–5.38%. In an investigation by Matoh et al. (1991), that the photosynthetic rate of single leaves was improved by increasing the concentration of Si in the leaf blade by the application of calcium silicate, and those authors noted that this improvement occurred because the leaf blade maintains the plant's functional water status. Ando et al. (2002) showed that the photosynthetic rate of the community of rice was increased by the application of calcium silicate, and the carbohydrate content in the rice grains was also increased. A later study by Fujii et al. (2008) revealed that paddy rice leaves rich in Si had an increase in carbohydrate content and a good ripening performance through an increase in photosynthetic rates to maintain the functional water status.

In 2010, Kaneda et al. (2010) demonstrated in a pot experiment that a rapid increase in leaf temperature in response to high-temperature treatment was suppressed by an application of calcium silicate from the ripening stage, and this application was also speculated to suppress the decrease in yield and improve the quality of brown rice by suppressing the consumption of carbohydrates accumulated in the leaf blade. In fact, Mori, and Fujii (2013) reported that the fructose, glucose, and sucrose content in the leaves after high-temperature treatment was 1.4 times higher with the application of calcium silicate compared to the control, suggesting that this change in content was a factor in reducing the decline in yield and quality. As mentioned above, we propose that the application of Si could be a promising soil fertilizer to control the degradation of yield and quality under high-temperature conditions.

The impact of high temperatures on the As concentration from a soil solution to the rice grain has recently been examined with high air temperatures after heading and elevated soil temperature (Neumann et al., 2017). Weber et al. (2010) reported the relationship between the increase or decrease in soil temperature and the leaching of arsenic into the soil solution as follows. When the soil temperature increased from 10 ◦C to >23 ◦C, As was rapidly released into the soil solution with an increase in microbial activity. In contrast, when the soil temperature was decreased from 23 ◦C to 14 ◦C or 5 ◦C, the As concentration was 10 times lower at 14 ◦C or 5 ◦C compared to that at 23 ◦C (Weber et al., 2010). Tyrovola et al. (2009) reported that the As concentration in soil solution kept at 40 ◦C was higher than those at 10 ◦C and 20 ◦C. Neumann et al. (2017) cultivated rice plants in California paddy soil packed into rhizome boxes, using different soil temperatures (26.1 ◦C and 30.5 ◦C), and synchrotron X-ray fluorescence (XRF) imaging at the root base of the rice plants showed that more iron (Fe and As were accumulated at 30.5 ◦C compared to 26.1 ◦C soil-temperature treatment). Regarding these results, Neumann et al. (2017) suggested that the increased soil temperature increased the activity of the iron-reducing bacteria and that the leaching of iron from the solid phase into the soil solution increased the leaching of As that was adsorbed by the iron in the soil.

Arao et al. (2018) recently reported multiyear data revealing that the daily mean high temperature during the 2–4 weeks post-heading was significantly correlated with the iAs concentrations in the rice grains, indicating that this period (2–4 weeks post-heading) is important in the uptake and accumulation of As in rice grains. Together the above-summarized studies demonstrated that high air and soil temperatures are the most important factors needed to release more As in the soil solution, and that these increased temperatures are related to the transfer of As from the rice root to grains and deposited more As in rice grains compared to the optimum temperature.

We observed herein that the average mean soil temperature was 25.7 ◦C in the ambient treatment in the TGCs, 26.9 ◦C in the mildly-high treatment, 27.5 ◦C in the moderately-high treatment, and 28.0 ◦C in the super-high temperature treatment (Table

3.1). Although we did not measure the concentration of As in the soil solution during the growing period, the concentration of As in the soil solution likely remained higher in the high-temperature treatments, as is evident from many of the above reports. Our experiment showed that the As concentrations of brown rice in the high-temperature treatments remained higher than that in the ambient treatment. In fact, with the use of the TGCs, the concentration of As in brown rice showed a significant positive correlation with the air and soil temperatures during the ripening period and was higher as the air and ground temperatures increased. Our findings thus support the relationship between air temperature during the ripening period and the brown rice As concentration suggested by Arao et al. (2018).

We also observed that the Si application had a significant effect on the As accumulation in brown rice grains (Table 3.3). Many researchers have reported that the application of Si materials is more beneficial and effective to reduce the As uptake and concentration in rice grains. Matsumoto et al. (2015a) cultivated rice plants in concrete frames in a field and applied calcium silicate treatment; they reported that this treatment effectively reduced the As concentration in the grain and straw compared to the control (without calcium silicate application). Bogdan, and Schenk (2008) showed that soils containing high levels of Si that is available to plants produce lower concentrations of As in rice plants and that the application of Si to the soil may reduce the concentration of As in rice. Moreover, Li et al. (2018) observed that the application of Si fertilizer to the soil reduced the total As of rice straw and grain by 78% and 16%, respectively. Even different sources of Si materials (i.e., Si-potash fertilizer, Si-Ca fertilizer, semi-finished product of Si-potash fertilizer, and Na2Sio3) showed reduced As concentrations in both straw and grain (Wang et al., 2016).

It has been reported that the similarity between the chemical forms of As and Si is an essential factor in considering the decrease in the As concentration of brown rice due to silicate application. Several research groups noted that the addition of Si significantly elevated the As concentration in soil solution, and that it even decreased the accumulation of As in some important aerial parts of the plant (stem, leaf, husk, and grain). Arsenic and Si are competitive elements for ferrihydrite and gothite (Swedlundi et al., 1999; Waltham et al., 2002; Luxton et al., 2006) because of their chemical similarities. The increase in As concentration in the soil solution caused by the addition of Si is the result of competitive adsorption between silicate and arsenite on soil particles that are rich in iron oxide (Li et al., 2018). The competitive adsorption to the binding sites also occurred in the iron plaque of rice roots (Li et al., 2018). Thus, the competition between As and Si at the root surface should not increase the absorption of As by rice, even if the concentration of As in the soil solution increases (Makino et al., 2016).

It has been pointed out that the competition between As and Si has a significant influence on the process of As uptake by rice. Ma et al. (2008) concluded that arsenite (which is the dominant species of iAs in rice) and Si are transported by the same transporters (i.e., Lsi1 and Lsi2) because of the chemical similarities between arsenite and Si (Ma et al., 2008). In the present study, the application of calcium silicate had a considerable effect on the concentration of As in brown rice grains, as shown in Table 3.3. The concentrations of the dominant As species (iAs and DMA) in brown rice were significantly decreased by the Si application compared to the control without Si application at all temperatures tested. Similar results were observed by Li et al. (2018), who also documented in their pot experiment using rice plants grown at the temperature range 22 ◦C–35 ◦C that an application of Si significantly reduced the total As concentration in brown rice grains (by 24.1%), whereas the Si had only a slight and non-significant effect on the plant biomass (Li et al., 2018). The decrease in As concentration could be due to the competition of Si in the arsenite uptake and transport in aerial tissues.

Several studies showed that Si may suppress the arsenite uptake in a direct competition for membrane transporters, especially Lsi2 (Ma et al., 2006; 2007; 2008). An increase in the Si concentration in the rice body by the application of silicate can thus affect and compete with As for the transportation, and therefore mitigate the As absorption in rice, which is related to decreasing As contents in rice straw, followed by a decreased As concentration in rice grains under the high-temperature conditions described herein. These findings indicate that the competitive As uptake reduction by the application of silicate was effective even under high-temperature conditions. In addition, the increase in biomass and brown rice yield by the application of calcium silicate may have contributed to the decrease of the concentration of As in brown rice. In other words, if the accumulation of carbohydrates in brown rice is increased, the concentration of As in brown rice is considered to be diluted and decreased. In this regard, it is necessary to examine the relationship between the accumulation of carbohydrates and As in brown rice because it has been clarified that iAs translocates to brown rice through a sieve tube as described above (Yamaji et al., 2014; Song et al., 2014). The cause of the decrease in brown rice yield due to high temperature is that the high temperature accelerates the aging of the leaf and panicle, and the ripening period is shortened (Kim et al., 2011). Therefore, the reason for the high concentration of As in brown rice in high-temperature conditions may be that the dilution of As due to the accumulation of carbohydrates was insufficient for the shortened ripening period. The reason the concentration of As in the Si treatment was lower than that in the control may also be that the ripening damage due to high temperature was less than that in the control. However, such considerations would require a detailed study of the translocation rates of carbohydrates and As to brown rice, respectively. The application of Si would thus reduce the risk of human As intake from rice cultivated under high temperature.

It is important to note that the amount of calcium silicate applied in this study was approx. 1.5 times the recommended standard in Japan. This was because the available Si content of the soil used in this study was lower than that of the average paddy field soil in Japan. In addition, to assess the risk of As in rice sold at public markets, we tested soil with relatively low As concentrations. These factors may have facilitated the effect of the Si application on the mitigation of growth inhibition and reduction of As concentration in brown rice under high-temperature conditions. It is therefore necessary to study the amounts of Si applied according to the As and available Si contents in the soil. Further investigations of agronomic methods are required in a variety of field conditions to determine all of the precise effects of increased temperatures.

Chapter 4

Increased Arsenic Concentration in Brown Rice due to insufficient dilution effect under High Temperatures During the Ripening Period and Reduced by the Application of Converted Furnace Slag

This chapter presents an assessment of the effects of high temperature on the arsenic accumulation in brown rice grain and reduced by the application of converted furnace slag (CFS). It summarizes results on the presence of arsenic and carbon in straw, panicle, internode, and node of the rice plants. It also summarizes the application of the correlation of CFS materials and arsenic concentration in brown rice grain during the ripening period under different levels of high temperatures conditions where conducting experiments at TGC.

Abstract

"Koshihikari" rice cultivar grown in Wagner pots (1/5000a) with uncontaminated soil that was used for high-temperature treatment in TGC set at four temperature levels; ambient, mildly-high temperature, moderate-high temperature, and super-high temperature from 1 week after heading until harvest. In the TGC, a range of mean air temperatures was observed in the range of 3.2 °C above the ambient temperature. In the daytime, highest temperature was detected in the range of 5.0 °C above the ambient temperature and was 3.5 times higher temperature compare to nighttime temperature. Therefore, excessive As may accumulated in brown rice grain in day time compare to night time temperature. There was a significant regression linearity was found between temperature and dry matter, thereby reduction of yield also significant regression linearity which followed drastically one fifth in super high temperature compared to ambient. The reduction in yield was significant considerably mitigated by the application of CFS materials. The concentration of As and As species (iAs, and total As) in brown rice was significant regression linearity with temperature although DMA tended to increase with high temperature. Even, the concentration of total As was significant in each parts of rice straw (internode 2, node 1 and node 2) where carbon concentration was not significant with the temperature. However, CFS application significantly reduced As concentration in brown rice and each parts of rice plants in all high temperature treatment. 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.

1. Introduction

There are many researchers have been taken technique to prevent growth inhibited in rice plants to reduce As concentration. For example, selecting good quality of varieties with low As concentration, water management for paddy fields, soil modifier to reduce As concentration with increasing yield etc. (Matsumoto et al., 2015a, b; Li et al., 2009a; Zhao et al., 2010a). Among of those techniques, using soil modifier is an available and comfortable practice for paddy cultivation because of as further more restriction in other techniques. It was reported that Fe and silicate modifier decreased As absorption in rice and increased grain yield (Matsumoto et al., 2015a, b; 2016; Li et al., 2009a; Liu et al., 2004; Seyfferth and Fendorf, 2012; Dhar et al., 2020). Some of reason is responsible to reduce As absorption in rice by Fe modifier: the increase of free iron oxide in the soil solution influenced to formation of iron plaque around the root surface and arsenite is oxidized to arsenate by Fe which is immobilized because of Fe had a high quality of affinity to arsenate (Liu et al., 2004). Thus, remaining of large part of Fe oxidized to reduce As absorption in rice grain through root (Matsumoto et al 2015a, b; 2016). Mei et al. (2012) found that at the tillering stage, Fe plaque formation was at highest level although its concentration decreased after due to Fe oxidized of transformation. Garnier et al. (2010) reported in their comprehensive field experiment that As concentration in roots and in the iron plaque rose to 1000-1500 mg kg^{-1} towards the middle of the rice growing season and then it decreased to ~300 mg kg^{-1} towards the end, followed of the maximum As concentration of grain was 0.58 ± 0.05 mg kg^{-1}. Under this critical situation and as according to the discussion of above that the As concentration in rice grains will increase due to the global temperature rise, and it will therefore be necessary to reduce the As concentration not only As contaminated soil but also in As uncontaminated soil.

However, temperature gradient chamber is appropriate and familiar technique for conducting experiment where provide markedly differences in air temperature in different location are critical in weather forecasting and climate changes in the global environment (Okada et al., 1995). Providing a continuous one-way airflow along the long axis of the TGC, the air temperature rises gradually with the distance from the inlet by sensible heat gain form solar radiation or supplementary heating cables as defined as ratio of the differences between temperature of 2 or 3 °C thermometer. On global and annual basis, the dynamics of the atmosphere can be understood to increase temperature in the TGC and how may affect on crop production, and thereby as attempting, be able to be taken to prevent critical situation against high-temperature. Nevertheless, in the production of paddy rice, the influence of temperature on the As aggregation and speciation of rice grains must be studied in order to consider the impact of temperature changes on the rice yield and the As speciation in the rice.

In our previous report, Si is investigated to reduce As concentration and increased brown rice grain yield even in high temperature condition although did not use of Fe materials application. Even though many studies have been already justified and proven that Fe modifier can increase tolerances not only As uncontaminated soil but also contaminated soil, and their impacts on As accumulation in rice grains, translocation, distribution in aerials parts in rice plants. Nevertheless, remain unclear of different types of Fe materials activities under high temperature conditions. Therefore, we decided to examine whether soil modifier of Fe that may be inhibited As absorption in brown rice grain even in high temperature conditions. Consequently, the study aimed of our present experiment that to determine the relationship during the ripening period between temperature and As concentration in brown rice in the TGCs where Fe modifiers were applied as soil modifier to reduce As concentration in brown rice in the uncontaminated soil.

2. Materials and Methods

2.1. Soil Preparation, Application of converted furnace slag, Plant materials and Rice cultivation

The present experiment was conducted at the area of Izumo, Shimane, Japan. We collected As-uncontaminated gray low layer paddy field land soil from Honju, Matsue, Shimane, Japan, which filled up of 3kg soil into the wagner pots (1/5000 a, Fujiwara Scientific, Tokyo). As a basal fertilizer, 2.8 g of a compound fertilizer that contained N 14%, P_2O_5 14%, and K_2O 14%, applied before the seedlings were transplanted. Pots were kept under natural condition before started of heat-treatment. We used two quantities of converted furnace slag (CFS, FM GOLD, Yoneda Industry Co., Okayama, Japan) which is generally used as Fe material in Japan. The CFS was used to apply at a rate of 15 g pot $^{-1}$ as CFS15 and 30 g pot $^{-1}$ as CFS30, respectively to reduce the uptake of As in brown rice grain. No converted furnace slag application was applied in the control pots to identify the basal condition of As accumulation in brown rice. The soil analyzed contained 1.6% total C, 0.14% total N, 1.2 mg kg^{-1} of available As (1 M HCl extractable form), and it had soil pH of 5.5. In keeping with local guidelines for yield growth, the rodents, diseases, and weeds were intensively regulated.

For rice cultivation a common Japanese cultivar (*Oryza sativa* L. cv. 'Koshihikari') has been chosen, and sterilized seeds were germinated at 32°C for 24 h. Seedlings of germinated seeds were sown in paper pots (comprised of 578 1.5-cm^2, 3-cm-deep blocks) on soil formulated for seedling better growth (Green soil, Izumo Green Co., Izumo, Japan). Seedlings of rice that were twenty-five days old was transplanted to Wagner pots at 22 May 2019. Three seedlings were kept flooding condition from the transplanting to until harvesting. A synthetic liquid fertilizer containing 0.21 per cent N as a component of $(NH_4)_2SO_4$ was added to each pot with 0.5 g of N at 48 days after the seedlings were transplanted. Until the start of the high-temperature treatments, the plants were cultivated outdoors.

2.2. High-Temperature Treatment

For high temperature treatments, a long 40-m and 5.4 m wide TGC (Figure 4.1) used to examined the As accumulation in rice grains and some of aerial parts in rice. To get the daily temperature variation of at day and night, the TGC was set up from entrance to deep following to the order: ambient, mildly-high, moderately-high, and super-high-temperature treatments at Izumo research center, Shimane, Japan. To detect the effect of the day and nighttime temperatures were calculated at 6:00-18:00 and 18:00-6:00, respectively in 4 temperature treatments. Each part of temperature section was set up by the appropriate temperature logger to understand the temperature variation and separating the borders of each treatment segment was not a translucent nylon curtain with vertical slits. To create warmer inside of TGC, three high quality and resolution of electric fan was set up at the end of back that continuously provided hot condition from mildy high to super temperature treatment block. The temperature in the marginally elevated plot is similar to the outside temperature (ambient plot) when the airflow travels through the aperture first and the temperature slowly decreases at the TGC's far end (Figure 4.2). The foundation of each TGC was built to have temperature changes from the entry to deep within the TGC, and the air and soil temperatures will be assumed to be in the following order: ambient< mildly-high< moderately-high < super-high. Through changing the exhaust, velocity of the fan the daytime and nighttime air temperature was adjusted to not be exceeded or reduced at 40 °C and 22 °C, respectively. The temperatures of the air were reported before harvest.

The heading date was July 31st when 50 percent of the overall heading happened. Seven days after the heading date (August 7th), twenty-four pots (eight control pots, eight CFS15-application pots and eight CFS30-application pots) were transferred to each sector of the four temperature treatments in TGC. To continue of this study, a total number of 96 pots (24×4) was used. All pots were kept for grown in the TGC until harvesting.

2.3. Collecting Plant Samples for chemical analysis and measurement

At maturity (September 10th) we harvested the rice plants from each pot for assessment of the As concentration in rice plant tissues. The collected samples of the plants were air-dried for 7 days in a greenhouse. Dividing the air-dried samples into straw and panicles. Husks and unfilled grains were removed for the analyses of the As and speciation concentrations in brown rice grains. Unfilled grains were removed by ammonium sulfate solution, and the grains were counted by a machine (#IC-1, Aidex Co., Nagoya, Japan). Filled grains were de-husked by a machine (#FC2K, Otake Agricultural Machinery Co., Aichi, Japan). To determine the specific biomass production, total dry weight, panicle dry weight, straw dry weight, panicle number, culm length, and panicle length were measured. Each straw of the sample was divided into internode I , node I, internode II, node II, and under inter-node Ⅲ as shown in Fig. 4.2. Samples of the nodes were cut 3.5 mm above and below the center of the node (total length 7 mm). Since the rice husks could not be recovered, they were not used in this analysis.

2.4. Chemical Analyses of the As, As Speciation and Carbon in the Plant Tissues

For the analysis of As Five ml of 5:1 (v/v) HNO_3/H_2O_2 was added to each powdered sample of brown rice (0.5 g), and the samples were wet-digested in a digestion system (Eco-pre system 24T, Actac, Tokyo). The concentrations of As in the degraded samples was determined by inductively coupled plasma mass spectrometry (ICP-MS) (Model 8800, Agilent, Hanover, Germany).

We determined the As speciation in the rice grains by using the method of Matsumoto et al. (2016). Powdered brown rice (0.5 g) was mixed with 2 mL of HNO_3 (0.15 M) in a 10-mL capped high-density polyethylene centrifuge tube, and the mixture was heated in an aluminum heating block at 80°C for 2 h. The obtained extract was diluted to

10 ml with water and passed through a 0.45-μm filter before analysis. The As speciation of diluted solution samples was determined by a high-performance liquid chromatography (HPLC)/ICP-MS system. A Super IC-Anion column (5 μm ID, 4.6 mm ID, 150 mm ID) and a guard column (Tosoh, Tokyo) equipped with an isocratic mobile phase system consisting of 10 mM ammonium acetate and an HPLC system (LC10A, Shimadz, Kyoto, Japan) were used. The injection volume and mobile-phase flow were set at 10 and 800 μL min^{-1}, respectively. Arsenic concentration was determined using the ICP-MS system (Model 8800, Agilent, Hanover, Germany). Total inorganic As (iAs) is expressed as the sum of arsenite and arsenate. The accuracy of the analysis was certified by the reference material (CRM) (rice flour, NMIJ CRM 7503-a: arsenite 0.0711 ± 0.0029 mg kg^{-1}, arsenite 0.0130 ± 0.0009 mg kg^{-1}, dimethyl arsenate [DMA] 0.0133 ± 0.0009 mg kg^{-1}; National Institute of Advanced Industrial Science and Technology [NIJM], Japan).
The concentration of total carbon in the powder samples was determined by the NC-80 AUTO (Sumika co. Ltd. Tokyo).

2.5. Statistical Analyses

The effects of temperature on the growth biomass production, As concentrations carbon concentration and those accumulations were validated by an analysis of covariance (ANCOVA) with air temperatures as a covariate. We also tested the significance of the control and CFS application when the ANCOVA was significant.

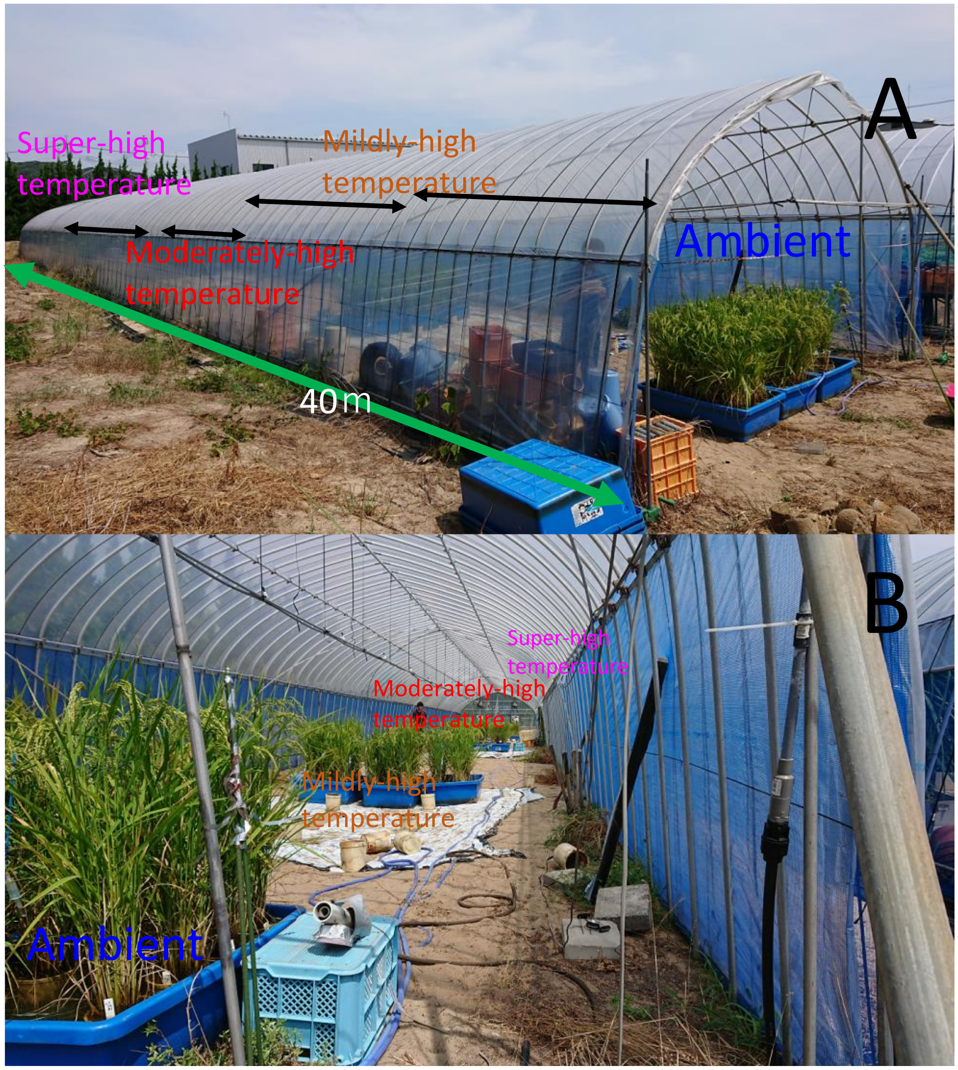


Figure 4.1. Temperature gradient chambers (TGCs) used for high-temperature treatment during the ripening period.

A: external appearance. B: inside the TGC.

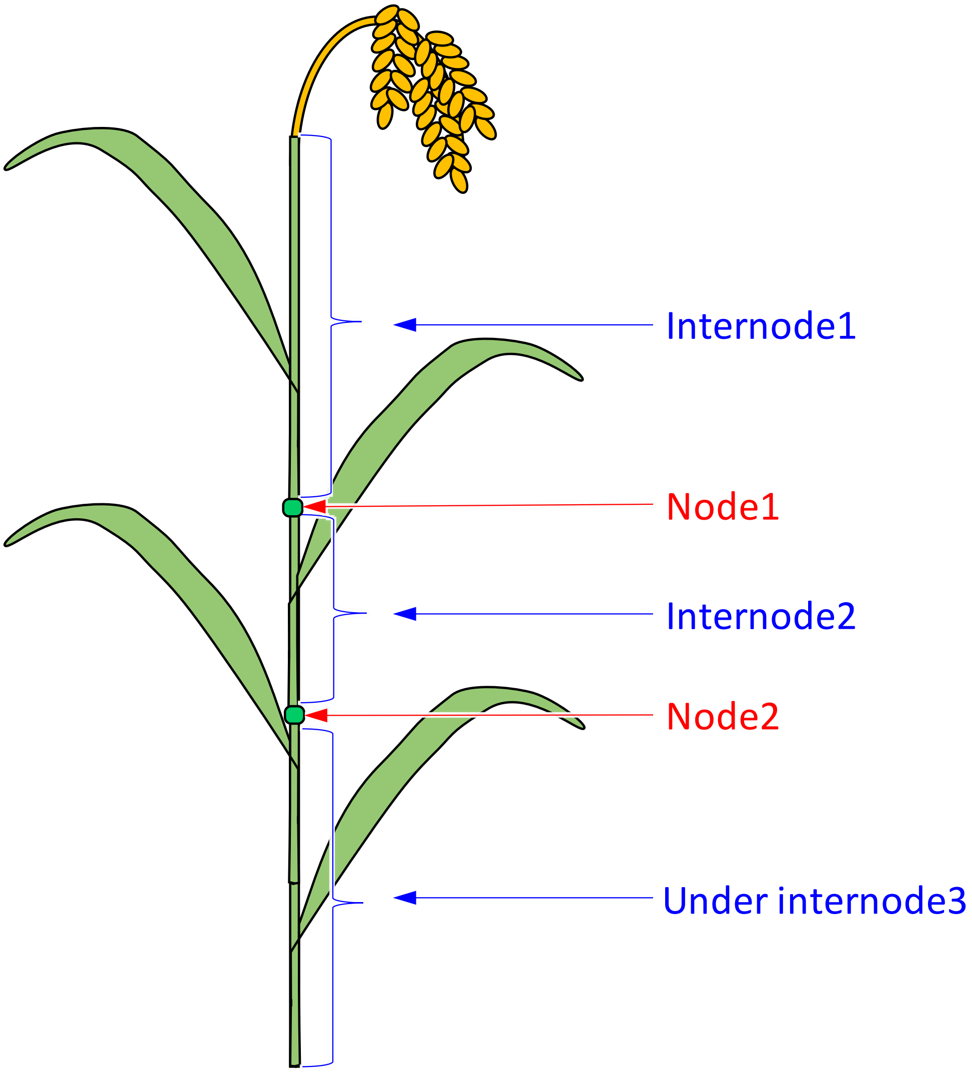


Figure 4.2. Schematic diagram of each part of rice divided for analysis.

3. Results

3.1. Temperature Differences between the Ambient, Mildly-High, Moderately High, and Super-High Treatments in the TGC During the Ripening Period

Figure 4.3 (A-E) indicates the average variations in air temperature between the four temperature treatments in the TGC for T_{mean}, T_{max}, and T_{min} during the ripening period. The disparity in average daily air temperatures among the four treatments (i.e., ambient, mildly-high, moderately-high, and super-high temperatures) was 1.7–3.2 °C (T_{mean}), 5.2–8.9 °C (T_{max}), and 0.9–1.6 °C (T_{min}), respectively, according to the regular temperature details. The average daily mean air temperature during the ripening period, as described in Table 4.1, was 26.5 °C in the ambient section of the TGC, 28.1 °C in the mildy high section, 29.6 °C in the moderately high section and 29.4 °C in the super-high section. Hence, the largest difference between the average daily mean temperatures observed during the ripening period in the daily temperature data was 3.1 °C above the ambient.

The average daily maximum air temperature was 30.7 °C in the ambient-temperature section, 35.8 °C in the mildly-high, and 38.8 °C in the moderately-high, and 39.6 °C in the super-high. Consequently, the average daily maximum air temperature difference was 8.0 °C between the four temperature treatments during the ripening period.

The average daily minimum air temperature was 22.7 °C in the ambient-temperature section, 23.6 °C in the mildly-high, and 24.4 °C in the moderately-high, and 23.7 °C in the super-high. The average daily minimum air temperature difference between the four temperature treatments during the ripening period was therefore 1.6 °C.

Figure 4.3 (D-E), displays the average air day time (6:00-18:00) and nighttime temperature differences in the TGC between the four temperature treatments (i.e., ambient, mildly-high, moderately-high, and super-high temperatures). The average daily daytime air temperature and nighttime temperature disparity among the four treatments (i.e., ambient, mildly high, moderately high, and super-high temperatures) was 2.8.–5.0 °C, and 0.7-1.4 °C, respectively, based on the standard temperature information. During the ripening period, the average daily mean day time air temperature as defined in Table 4.1 was 28.1 °C in the TGC ambient section, 31.0 °C in the mildly-high section, 33.1 °C in the moderate-high section and 33.2 °C in the super-high section. The average daily mean nighttime air temperature during the ripening period, as described in Table 4.1, was 24.8 °C in the ambient section of the TGC, 25.5 °C in the mildly-high section, 26.2 °C in the moderately high section and 25.6 °C in the super-high section.

Basis on the daily mean, minimum, maximum, daytime and nighttime temperature data, there were clear temperature differences between the four high-temperature treatments. Even, maximum temperature was too high in super high as we expected. We thus observed an effective and meaningful air temperature difference between the ambient-temperature sections of the TGC and the three high-temperature treatments.

3.2. Yield and yield components

The yield of brown rice was slightly higher in the mildly-high temperature treatment than in the ambient, but it decreased in the moderately- and super-high temperature treatment due to the increase in temperature, and the yield in the super-high temperature treatment was drastically reduced to about one-fifth of that in the ambient (Table 4.2, Table 4S1). Regarding the relationship between air temperature and yield during the ripening period, significant regression linearity was observed in daytime temperature, nighttime temperature, daily mean temperature, daily maximum temperature, and daily minimum temperature. The p-value with the highest relationship was shown with the daily maximum temperature.

No significant linearity of regression with temperature was observed for the panicle number, grain number per panicle, culm length, and panicle length, and no effect of high temperature was observed for these measurement items (Table 4.2, Table 4S1).

Since the best regression linearity was observed in yield with the mean of the maximum temperature during the ripening period, the mean of the maximum temperature was used as a covariate to perform an analysis of covariance for brown rice yield, panicle number, grain number per panicle, culm length, and panicle length to investigate the effect of soil amendments under different temperature conditions. There was no significant difference in the application of soil amendments to the brown rice yield. In contrast, the grain number per panicle tended to increase with CFS application compared to the control, and there was a significant difference between CFS30 and the control. It was also shown that CFS application resulted in significantly shorter culm length and significantly longer panicle length.

3.3. Dry matter weight by parts in rice plant

The dry weight of brown rice showed a significant linearity of regression with temperature, as shown in Table 4.2 of the brown rice yields, and decreased with increasing temperature, with the most apparent relationship with maximum temperature (Table 4.3, Table 4S2). There was no significant linearity of regression between dry matter weight and air temperature in internode 1, internode 2, node 1, and node 2. On the other hand, there was a significant linearity of regression between dry matter weight and temperature in straw under node 2, and the dry weight of that increased with increasing temperature. As a result, there was a significant linearity in the dry matter weight and temperature in the dry weight of the whole stem, with the lowest p-value of daily maximum temperature. An analysis of covariance with maximum temperature as a covariate and application of materials as a fixed factor showed no significant effect of material application in any treatments, and there was no difference between the treatments.

3.4. Concentration of As in brown rice

The concentration of iAs in brown rice was lowest in the ambient and increased by high-temperature treatment (Table 4.4, Table 4S3). Significant linearity of regression between temperature and iAs concentration was observed at all temperatures. The relationship between maximum temperature and iAs concentration had the smallest p-value. In contrast, the DMA tended to increase with high-temperature treatment up to the medium and high-temperature ranges but decreased slightly in the super high-temperature treatment, and the linearity of the regression was not significant. Similar to iAs, the total As concentration increased by high-temperature treatment, significant regression linearity was observed, and the p-value of the maximum temperature was the smallest.

Therefore, analysis of covariance was performed with the maximum temperature as the covariate and the material application as a fixed factor, and the effect of the material application under different temperatures was verified. The effect of CFS application on the iAs in brown rice was significant, and a significant difference was observed between the application of CFS30 and the control (Table 4.4). However, the effect of CFS application on DMA was not significant. The application of CFS in the total As concentration showed a significant effect, and it decreased as the CFS application rate increased, showing a significant difference from the control.

3.5. Concentration of total As and carbon in each parts of rice straw

We examined the relationship between As concentration and temperature in each part of the straw and the effect of applying CFS. The total As concentration tended to decrease with the increasing temperature in the internode 1 and straw under node 2, but the linearity of the regression was not significant (Table 4.5, Table 4S4). On the other hand, the total As concentration in the node 1, internode 2 and node 2 showed significant regression linearity with temperatures. Especially in node 2, extremely high linearity was observed, and the total As concentration decreased as the temperature decrease. When these significant linearity were shown, the correlation between the concentration and the maximum temperature and the average daytime temperature was high. Covariance analysis was conducted with maximum temperature as a covariate and application of soil amendment material as a fixed factor, and the application of CFS had a significant effect on total As concentration at all parts, with CFS30 showing the lowest total As concentration.

We focused on the part-specific carbon concentration as an indicator of translocation through the sieve tube. The relationship between temperature and carbon concentration in brown rice and straw is shown in Table 4.6. There was no significant linear relationship between temperature and carbon concentration at any of the parts. On the other hand, there was a significant effect of the material application on carbon concentration in the internode1, internode 2, and straw under node 2, and the application of CFS significantly reduced the carbon concentration. There was no significant effect of the material application on the carbon concentration of brown rice.

3.6. Accumulation of total arsenic and carbon in each parts of rice plant

Since the As concentration and dry matter weight had the highest correlation with the maximum temperature, the linearity of the As accumulation with the maximum temperature was examined. The total As accumulation in the brown rice was significantly reduced with increasing temperature in the moderately- and super- high temperature treatments, although it was highest in the mildly-high-temperature treatment where the dry matter weight was high (Table 4.7, Table 4S6). This may be due to a decrease in the production of brown rice dry matter at higher temperatures. In straw, there was a meaningful decrease in the total As accumulation in the nodes and a significant negative linear regression between temperature and As accumulation in the node 2. Although not significant at the 5% level in node 1, the p-value was 0.061, showing a significant linearity at the 10% level, indicating that As accumulation decreased at higher temperatures. When the effect of the material application was verified using the maximum temperature as a covariate, the As accumulation in each part was reduced by application of CFS30, suggesting that the application of CFS suppressed the absorption of As.

Similar to the total As accumulation, the carbon accumulation in brown rice showed a linearity of regression with the maximum temperature, and the carbon accumulation decreased as the temperature increased (Table 4.8, Table 4S7). There was no significant linear relationship between maximum temperature and carbon accumulation in the internode 1, node 1 and node 2. However, there was a significant linearity of regression in the internode 2, with carbon accumulation increasing with increasing temperature. In addition, although not significant at the 5% level, carbon accumulation tended to increase with increasing temperature even in the straw under node 2. In the case of the total amount of straw, a significant linear relationship was observed between the maximum temperature and the amount of carbon accumulated, indicating that the amount of carbon accumulated in straw increases as the temperature increase. When the effect of the material application was verified using the maximum temperature as a covariate, it was found that the application of CFS30 significantly decreased the accumulation of carbon in the internode 1. However, in all other parts, the application of the material did not show a significant effect for the amount of carbon accumulation.

3.7. Distribution of As and carbon in each parts of rice plant

The distribution ratio of As was calculated based on the amount of As accumulated in each part of the rice plant, and the effects of temperature and material application were investigated. As for the effect of temperature at each part of the rice plant (Fig. 4.4A), the distribution ratio of As to brown rice was similar between ambient and mildly-high temperature treatment. The ratio of As decreased significantly with increasing temperature in the moderately-high and super-high temperature treatment. On the other hand, internode 1, node 1, internode 2, and node 2 had similar As distribution ratios in the temperature treatments, and no effect of temperature was observed. However, the distribution ratio of As to under internode 3 increased with increasing temperature compared to the ambient and mildly-high temperature treatment, and As tended to remain in this part. There was no clear difference among the material application (Fig. 4.4B), suggesting that there was no effect of the material applied on the distribution rate of As in the rice plant.

The effects of temperature and material application were investigated by calculating the distribution rate based on the amount of carbon accumulation in each part of the rice plant. Regarding the effect of temperature at each part (Fig. 4.5A), the distribution ratio of carbon to brown rice was the highest in the ambient, and the distribution ratio decreased significantly as the temperature increased. The highest carbon distribution ratio to brown rice was observed in the ambient, and the distribution ratio decreased markedly with increasing temperature. The carbon distribution ratio to the nodes was almost 1% or less in all conditions. Contrary to brown rice, the carbon distribution ratio of straw (internode 1, 2 and under internode 3) tended to increase with increasing temperature, suggesting that the high temperature suppressed carbon translocation to brown rice from straw, and remained in the straw. Regarding the effect of material application at each part of the rice plant (Fig. 4.5B), there was no apparent difference in carbon distribution ratio between the control and CFS15, but the application of CFS30 showed a higher distribution ratio to brown rice and a trend toward a lower carbon distribution ratio in the internode 1 and the under internode 3 compared to the control.

3.8. Changes in the distribution ratio of As and carbon in rice plant

The effect of temperature on the distribution rate of As and carbon was investigated by comparing the indexes of the distribution ratio of As and carbon in each high-temperature treatment when the ambient was set at 100 (Table 4.9). The distribution ratio of As tended to increase with increasing temperature in the straw under internode 3. On the other hand, the distribution ratio of As in the other parts of the straw did not show any trend. The carbon distribution ratio tended to increase with the increase in temperature in all parts of the straw. The distribution ratios of As and carbon to brown rice both decreased with increasing temperature, but the ratio of carbon decreased at a greater rate than that of As.

A negative correlation was found between changes in the carbon distribution ratio and As concentration in brown rice (Fig. 4.6), indicating that the concentration of As in both inorganic and total As increased as the carbon distribution ratio of brown rice decreased due to an increase in temperature.

The As/carbon ratio of the distribution ratio was calculated to compare the degree of influence of the temperature change on the change of the distribution ratio between As and carbon. There was no change in the As/carbon distribution ratio due to high temperature in the straws under internode 3, but the As/carbon ratio tended to decrease with increasing temperature in all other straw parts (Table 4.10). On the other hand, the As/carbon ratio of the distribution ratio in brown rice tended to increase with increasing temperature.

It was found that the As/carbon ratio of the distribution ratio to brown rice increased due to high temperature, and the concentration of total As and iAs in brown rice increased (Figure 4.7). In other words, it was considered that a decrease in the carbon distribution ratio to As increased the brown rice As concentration.

In Fig. 4.7, it was clarified that the distribution ratio of As and carbon in brown rice had a large effect on the concentration of As in brown rice, so the effect of temperature on this As/carbon distribution ratio was examined. That is, the As/carbon distribution ratio was increased with temperature (Figure 4.8). These results revealed that high temperature promotes a decrease in the carbon distribution rate more than a decrease in the As distribution rate in brown rice.

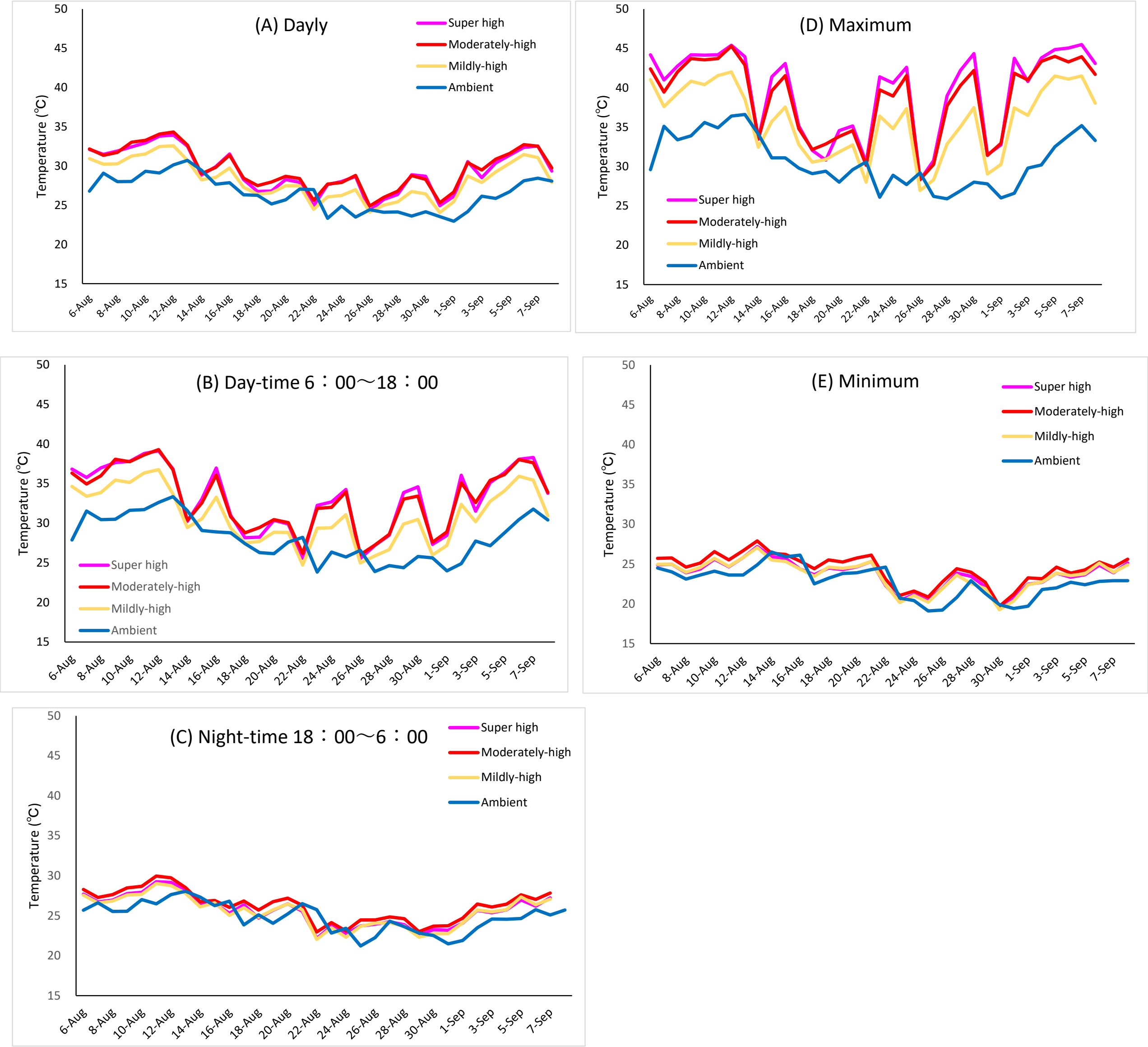


Figure 4.3. Changes in average daily mean air temperature (A), day-time mean air temperature (B), night-time mean air temperature (C), daily maximum air temperature (D), and daily minimum air temperature (E) during the ripening period.

Table 4.1. Average air temperatures during the ripening period

Temperature treatment	Day	Day-time (6:00-18:00)	Night-time (6:00-18:00)	Maximum	Minimum
Ambient	26.5	28.1	24.8	30.7	22.7
Mildly-high	28.2	31.0	25.5	35.8	23.6
Moderately-high	29.6	33.1	26.2	38.8	24.4
Super high	29.4	33.2	25.6	39.6	23.7

Table 4.2. Temperature effect on yield and yield component

Main factor		Treatment	Brown rice yeild (g pot^{-1})	Panicle number (pot^{-1})	Grain number (Pancle^{-1})	Culm length (cm)	Panicle length (cm)
Temperature		Ambient	20.2 ± 1.0	20.3 ± 1.0	62.9 ± 3.1	52.6 ± 1.4	22.0 ± 1.7
		Mildly-haigh	21.2 ± 1.0	21.7 ± 0.7	65.1 ± 2.2	57.2 ± 1.8	20.3 ± 1.5
		Moderately-high	14.1 ± 0.9	20.0 ± 1.1	66.2 ± 1.9	52.9 ± 2.2	22.2 ± 1.6
		Super-high	4.0 ± 0.6	20.1 ± 1.5	66.2 ± 2.0	54.5 ± 2.4	22.6 ± 1.3
Material application		Control	14.4 ± 1.7	22.0 ± 0.9	61.2 ± 1.7 a	59.8 ± 1.1 a	16.4 ± 0.4 a
		CFS15	13.8 ± 2.2	19.7 ± 0.9	64.5 ± 2.1 ab	50.9 ± 1.4 b	24.7 ± 0.8 b
		CFS30	16.4 ± 2.5	19.9 ± 1.0	69.6 ± 1.4 b	52.1 ± 1.5 b	24.3 ± 0.8 b
Ancova	Significance of regression	Day time	p = 5.9E-06 ****	p = 0.743	p = 0.183	p = 0.714	p = 0.549
		Night time	p = 0.027 *	p = 0.780	p = 0.229	p = 0.933	p = 0.781
		Dayly	p = 4.4E-05 ****	p = 0.745	p = 0.184	p = 0.755	p = 0.584
		Maximum	p = 3.3E-06 ****	p = 0.783	p = 0.184	p = 0.614	p = 0.589
		Minimum	p = 0.036 *	p = 0.842	p = 0.224	p = 0.803	p = 0.884
	Material application		p = 0.524	p = 0.177	p = 0.006 **	p = 7.3E-05 ****	p = 5.1E-10 ****

Different letters within a column of material application indicate significant differences by Tukey test ($p<0.05$)

The p-values for material application are for the case maximum temperature as a covariate

* Represents significance at the 0.05 probability level.

**Represents significance at the 0.01 probability level.

***Represents significance at the 0.001 probability level.

****Represents significance at the 0.0001 probability level.

Table 4.3. Temperature effect on dry matter weight by parts in rice plant

Main factor		Treatment	Brown rice (g pot^{-1})	Internode 1 (g pot^{-1})	Node1 (g pot^{-1})	Internode 2 (g pot^{-1})	Node 2 (g pot^{-1})	Under node3 (g pot^{-1})	Sum of stem (g pot^{-1})
Temperature		Ambient	17.2 ± 0.8	6.1 ± 0.3	0.32 ± 0.02	6.1 ± 0.3	0.48 ± 0.03	8.5 ± 0.5	21.5 ± 0.9
		Mildly-haigh	18.0 ± 0.8	6.9 ± 0.3	0.36 ± 0.01	7.1 ± 0.2	0.60 ± 0.03	9.9 ± 0.6	24.9 ± 0.7
		Moderately-high	12.0 ± 0.7	6.3 ± 0.3	0.32 ± 0.03	6.7 ± 0.4	0.52 ± 0.06	10.4 ± 0.7	24.3 ± 1.3
		Super-high	3.4 ± 0.5	6.1 ± 0.2	0.34 ± 0.02	6.5 ± 0.6	0.51 ± 0.04	10.9 ± 0.9	24.3 ± 1.0
Material application		Control	12.3 ± 1.5	6.7 ± 0.2	0.31 ± 0.01	7.2 ± 0.2	0.51 ± 0.03	9.5 ± 0.6	24.1 ± 0.6
		CFS15	11.7 ± 1.8	6.0 ± 0.2	0.34 ± 0.02	6.4 ± 0.4	0.54 ± 0.03	10.6 ± 0.7	23.8 ± 0.9
		CFS30	13.9 ± 2.1	6.4 ± 0.3	0.36 ± 0.03	6.2 ± 0.4	0.53 ± 0.05	9.8 ± 0.6	23.3 ± 1.2
Ancova	Significance of regression	Day time	$p = 5.9E-06$ ****	$p = 0.985$	$p = 0.806$	$p = 0.321$	$p = 0.681$	$p = 0.013$ *	$p = 0.046$ *
		Night time	$p = 0.027$ *	$p = 0.721$	$p = 0.972$	$p = 0.225$	$p = 0.584$	$p = 0.048$ *	$p = 0.064$
		Dayly	$p = 4.4E-05$ ****	$p = 0.933$	$p = 0.838$	$p = 0.293$	$p = 0.657$	$p = 0.015$ *	$p = 0.045$ *
		Maximum	$p = 3.3E-06$ ****	$p = 0.936$	$p = 0.733$	$p = 0.309$	$p = 0.639$	$p = 0.012$ *	$p = 0.040$ *
		Minimum	$p = 0.036$ *	$p = 0.607$	$p = 0.884$	$p = 0.186$	$p = 0.501$	$p = 0.044$ *	$p = 0.064$
	Material application		$p = 0.483$	$p = 0.207$	$p = 0.178$	$p = 0.097$	$p = 0.821$	$p = 0.414$	$p = 0.788$

Different letters within a column of material application indicate significant differences by Tukey test (p<0.05)

The p-values for material application are for the case maximum temperature as a covariate

* Represents significance at the 0.05 probability level.

**Represents significance at the 0.01 probability level.

Table 4.4. Concentration of As in brown rice

Main factor		Treatment	Inorganic As (m g kg^{-1})	DMA (m g kg^{-1})	Total As (m g kg^{-1})
Temperature		Ambient	0.298 ± 0.006	0.122 ± 0.014	0.472 ± 0.023
		Mildly-haigh	0.312 ± 0.008	0.163 ± 0.030	0.527 ± 0.032
		Moderately-high	0.333 ± 0.007	0.178 ± 0.011	0.543 ± 0.026
		Super-high	0.393 ± 0.010	0.156 ± 0.028	0.576 ± 0.019
Material application		Control	0.353 ± 0.012 a	0.176 ± 0.023	0.586 ± 0.026 a
		CFS15	0.335 ± 0.013 a	0.135 ± 0.010	0.525 ± 0.014 b
		CFS30	0.314 ± 0.011 b	0.154 ± 0.022	0.478 ± 0.020 b
Ancova	Significance of regression	Day time	*p* = 3.5E-06 ****	*p* = 0.121	*p* = 0.001 **
		Night time	*p* = 0.0255 *	*p* = 0.081	*p* = 0.020 *
		Dayly	*p* = 3.09E-05 ****	*p* = 0.105	*p* = 0.002 **
		Maximum	*p* = 1.18E-06 ****	*p* = 0.128	*p* = 0.001 **
		Minimum	*p* = 0.0277 *	*p* = 0.075	*p* = 0.018
	Material application		*p* = 0.011 *	*p* = 0.326	*p* = 5.8E-04 ****

Different letters within a column of material application indicate significant differences by Tukey test (p<0.05)

The p-values for material application are for the case maximum temperature as a covariate

* Represents significance at the 0.05 probability level.

**Represents significance at the 0.01 probability level.

***Represents significance at the 0.001 probability level.

****Represents significance at the 0.0001 probability level.

Table 4.5. Concentration of total As in the each part of rice straw.

Main factor		Treatment	Internode 1 (m g kg^{-1})	Node1 (m g kg^{-1})	Internode 2 (m g kg^{-1})	Node 2 (m g kg^{-1})	Under node3 (m g kg^{-1})
Temperature		Ambient	6.4 ± 0.4	22.6 ± 1.7	4.6 ± 0.2	18.4 ± 0.9	3.7 ± 0.3
		Mildly-haigh	6.8 ± 0.6	20.9 ± 1.5	4.0 ± 0.3	16.7 ± 1.1	3.3 ± 0.2
		Moderately-high	5.7 ± 0.4	19.2 ± 1.7	4.0 ± 0.4	14.6 ± 1.3	3.4 ± 0.2
		Super-high	5.5 ± 0.5	19.1 ± 1.9	3.6 ± 0.2	14.2 ± 1.3	3.2 ± 0.3
Material application		Control	6.0 ± 0.3 ab	24.4 ± 1.2 a	3.7 ± 0.2 a	19.5 ± 0.9 a	3.4 ± 0.1 ab
		CFS15	7.1 ± 0.4 a	20.2 ± 1.1 b	4.8 ± 0.3 b	14.6 ± 0.7 b	3.8 ± 0.3 a
		CFS30	5.1 ± 0.3 b	16.7 ± 1.3 b	3.6 ± 0.2 a	13.9 ± 0.9 b	2.9 ± 0.2 b
Ancova	Significance of regression	Day time	$p =$ 0.070	$p =$ 0.036 *	$p =$ 0.012 *	$p =$ 1.9E-04 ****	$p =$ 0.178
		Night time	$p =$ 0.160	$p =$ 0.066	$p =$ 0.071	$p =$ 0.002 **	$p =$ 0.320
		Dayly	$p =$ 0.078	$p =$ 0.037 *	$p =$ 0.016 *	$p =$ 2.3E-04 ****	$p =$ 0.194
		Maximum	$p =$ 0.080	$p =$ 0.037 *	$p =$ 0.009 **	$p =$ 2.0E-04 ****	$p =$ 0.163
		Minimum	$p =$ 0.197	$p =$ 0.067	$p =$ 0.061	$p =$ 0.002 **	$p =$ 0.298
	Material application		$p =$ 6.5E-04 ****	$p =$ 1.3E-04 ****	$p =$ 1.1E-04 ****	$p =$ 3.0E-06 ****	$p =$ 0.027 *

Different letters within a column of material application indicate significant differences by Tukey test (p<0.05)

The p-values for material application are for the case maximum temperature as a covariate

* Represents significance at the 0.05 probability level.

**Represents significance at the 0.01 probability level.

***Represents significance at the 0.001 probability level.

Table 4.6. Concentration of carbon in each parts of rice plant

Main factor		Treatment	Iinter node 1 (g kg^{-1})	Node1 (g kg^{-1})	Inter node 2 (g kg^{-1})	Node 2 (g kg^{-1})	Under node3 (g kg^{-1})	Brown rice (g kg^{-1})
Temperature		Ambient	393.5 ± 3.3	368.4 ± 3.6	403.6 ± 4.3	392.5 ± 1.5	405.3 ± 4.7	413.2 ± 2.7
		Mildly-haigh	395.0 ± 4.0	376.1 ± 1.3	404.7 ± 4.8	389.0 ± 1.4	407.2 ± 3.4	415.0 ± 2.0
		Moderately-high	397.6 ± 5.2	372.2 ± 2.7	408.0 ± 4.3	389.9 ± 1.3	403.3 ± 5.0	412.9 ± 1.7
		Super-high	396.0 ± 2.4	364.3 ± 9.0	408.3 ± 5.2	389.5 ± 1.5	399.6 ± 4.8	409.5 ± 1.0
Material application		Control	401.9 ± 2.1 a	372.2 ± 1.0 a	417.9 ± 2.3 a	388.6 ± 1.3 a	418.6 ± 1.3 a	414.1 ± 1.4 a
		CFS15	401.2 ± 2.1 a	364.9 ± 6.6 a	406.2 ± 2.3 b	389.7 ± 0.9 a	399.4 ± 2.9 b	410.2 ± 1.7 a
		CFS30	383.4 ± 2.1 b	373.6 ± 3.7 a	394.3 ± 3.6 c	392.4 ± 1.4 a	393.5 ± 2.2 b	413.7 ± 1.9 a
Ancova	Significance of regression	Daytime	$p = 0.265$	$p = 0.860$	$p = 0.236$	$p = 0.132$	$p = 0.175$	$p = 0.327$
		Night time	$p = 0.227$	$p = 0.707$	$p = 0.307$	$p = 0.192$	$p = 0.474$	$p = 0.785$
		Dayly average	$p = 0.248$	$p = 0.945$	$p = 0.240$	$p = 0.136$	$p = 0.211$	$p = 0.393$
		Maximum	$p = 0.283$	$p = 0.845$	$p = 0.243$	$p = 0.119$	$p = 0.173$	$p = 0.313$
		Minimum	$p = 0.233$	$p = 0.657$	$p = 0.318$	$p = 0.163$	$p = 0.517$	$p = 0.833$
	Material application		$p = $ 2.4E-07 ****	$p = 0.345$	$p = $ 5.3E-06 ****	$p = 0.084$	$p = $ 9.5E-09 ****	$p = 0.200$

Different letters within a column of material application indicate significant differences by Tukey test ($p<0.05$)

The p-values for material application are for the case maximum temperature as a covariate

* Represents significance at the 0.05 probability level.

**Represents significance at the 0.01 probability level.

***Represents significance at the 0.001 probability level.

Table 4.7. Accumulation of total As in each parts of rice Plant.

Main factor	Treatment	Brown rice (μ g pot^{-1})	Internode 1 (μ g pot^{-1})	Node 1 (μ g pot^{-1})	Internode 2 (μ g pot^{-1})	Node 2 (μ g pot^{-1})	Under internode 3 (μ g pot^{-1})	Sum of straw (μ g pot^{-1})	SUM (μ g pot^{-1})
Temperature	Ambient	9.5 ± 0.6	38.2 ± 1.9	7.1 ± 0.5	27.5 ± 1.6	8.8 ± 0.6	31.4 ± 3.1	112.9 ± 6.3	122.4 ± 6.4
	Mildly-haigh	11.1 ± 0.6	46.5 ± 3.7	7.5 ± 0.4	28.0 ± 1.7	9.9 ± 0.6	33.2 ± 3.0	125.0 ± 7.8	136.0 ± 8.1
	Moderately-high	7.5 ± 0.4	35.7 ± 3.3	5.9 ± 0.5	26.9 ± 3.2	7.2 ± 0.5	35.0 ± 3.3	110.7 ± 8.4	118.3 ± 8.4
	Super-high	2.3 ± 0.4	33.1 ± 2.9	6.3 ± 0.6	23.7 ± 2.6	7.1 ± 0.7	34.5 ± 4.1	104.7 ± 8.1	107.0 ± 8.3
Material application	Control	8.3 ± 1.0	39.8 ± 2.3 a	7.5 ± 0.4 a	26.4 ± 0.9 ab	9.8 ± 0.5 a	32.8 ± 2.7	116.2 ± 3.7 ab	124.4 ± 3.8 ab
	CFS15	7.1 ± 1.1	43.1 ± 3.1 b	6.8 ± 0.4 ab	30.8 ± 2.3 b	7.9 ± 0.5 ab	38.5 ± 2.6	127.1 ± 6.2 a	134.2 ± 7.0 a
	CFS30	7.5 ± 1.1	32.3 ± 2.5 a	5.8 ± 0.5 b	22.4 ± 2.0 b	7.1 ± 0.5 b	29.2 ± 2.7	96.7 ± 7.1 b	104.2 ± 7.6 b
Ancova									
	Significance of regression	p = 1.6E-04 ****	p = 0.186	p = 0.061	p = 0.348	p = 0.009 **	p = 0.379	p = 0.403	p = 0.181
	Material application	p = 0.621	p = 0.020 *	p = 0.015 *	p = 0.010 *	p = 0.001 **	p = 0.062	p = 0.003 **	p = 0.006 **

Different letters within a column of material application indicate significant differences by Tukey test ($p<0.05$)

The p-values for material application are for the case maximum temperature as a covariate

* Represents significance at the 0.05 probability level.

**Represents significance at the 0.01 probability level.

***Represents significance at the 0.001 probability level.

****Represents significance at the 0.0001 probability level.

Table 4.8. Accumulation of total carbon in each parts of rice.

Main factor	Treatment	Brown rice (g pot^{-1})	Internode 1 (g pot^{-1})	Node1 (g pot^{-1})	Internode 2 (g pot^{-1})	Node 2 (g pot^{-1})	Under internode 3 (g pot^{-1})	Sum of straw (g pot^{-1})	SUM (g pot^{-1})
Temperature	Ambient	7.1 ± 0.4	2.4 ± 0.1	0.118 ± 0.008	3.4 ± 0.2	0.189 ± 0.013	8.7 ± 0.3	14.8 ± 0.6	21.9 ± 0.9
	Mildly-haigh	7.5 ± 0.4	2.8 ± 0.1	0.136 ± 0.005	4.0 ± 0.2	0.232 ± 0.011	10.2 ± 0.3	17.4 ± 0.6	24.8 ± 0.8
	Moderately-high	4.9 ± 0.3	2.7 ± 0.2	0.120 ± 0.011	4.3 ± 0.3	0.204 ± 0.024	9.8 ± 0.6	17.1 ± 1.0	22.1 ± 1.2
	Super-high	1.4 ± 0.2	2.6 ± 0.2	0.123 ± 0.010	4.5 ± 0.4	0.198 ± 0.015	9.7 ± 0.4	17.1 ± 0.8	18.4 ± 0.8
Material application	Control	5.1 ± 0.6	2.9 ± 0.1 a	0.114 ± 0.004	4.0 ± 0.2	0.198 ± 0.011	10.1 ± 0.3	17.3 ± 0.5	22.4 ± 0.6
	CFS15	4.8 ± 0.8	2.6 ± 0.1 ab	0.124 ± 0.006	4.3 ± 0.3	0.211 ± 0.013	9.5 ± 0.4	16.7 ± 0.7	21.5 ± 0.9
	CFS30	5.8 ± 0.9	2.4 ± 0.1 b	0.134 ± 0.011	3.9 ± 0.2	0.209 ± 0.020	9.2 ± 0.5	15.8 ± 0.9	21.6 ± 1.4
Ancova									
	Significance of regression	$p =$ 4E-06 ****	$p =$ 0.271	$p =$ 0.852	$p =$ 0.009 **	$p =$ 0.722	$p =$ 0.068	$p =$ 0.032 *	$p =$ 0.143
	Material application	$p =$ 0.446	$p =$ 0.039 *	$p =$ 0.208	$p =$ 0.420	$p =$ 0.795	$p =$ 0.210	$p =$ 0.291	$p =$ 0.792

Different letters within a column of material application indicate significant differences by Tukey test (p<0.05)

The p-values for material application are for the case maximum temperature as a covariate

* Represents significance at the 0.05 probability level.

**Represents significance at the 0.01 probability level.

***Represents significance at the 0.001 probability level.

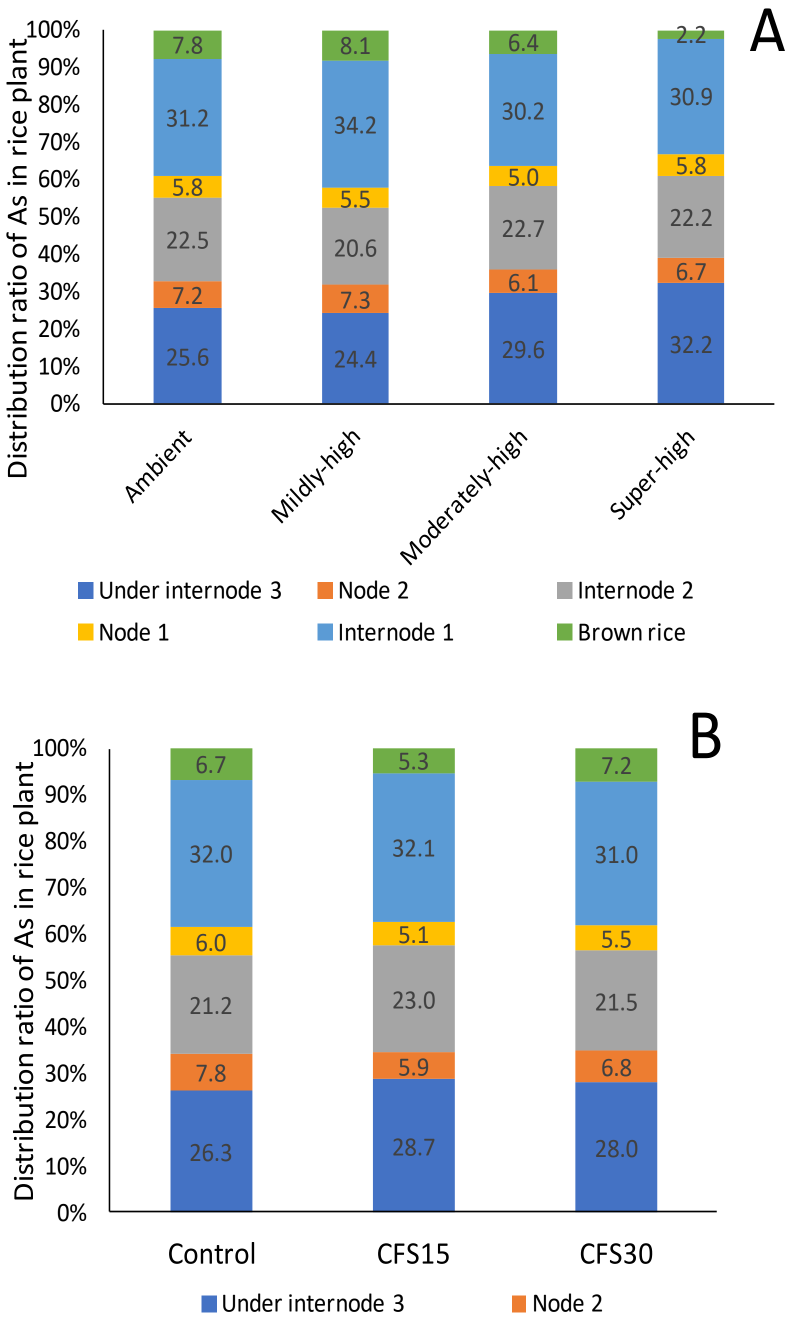


Figure 4.4. Effects of temperature in the ripening period (A) and application of CFS (B) in a distribution ratio of As in rice plant.

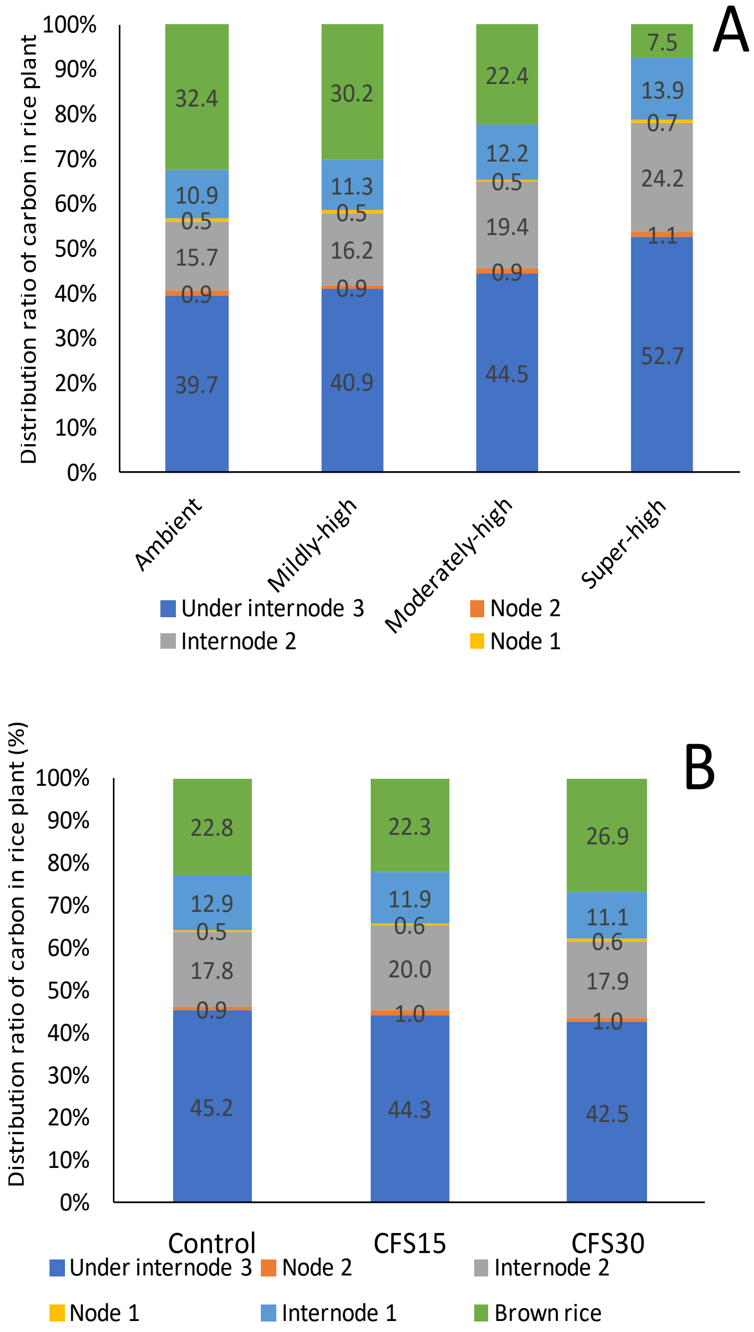


Figure 4.5. Effects of temperature in the ripening period (A) and application of CFS (B) in a distribution ratio of carbon in rice plant.

Table 4.9. The indexes of the distribution of As and carbon in each high temperature treatment when the ambient was set at 100.

Temperature treatment	As						Carbon					
	Under internode 3	Node 2	Internode 2	Node 1	Internode 1	Brown rice	Under internode 3	Node 2	Internode 2	Node 1	Internode 1	Brown rice
Ambient	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Mildly-haigh	95.3	101.5	91.5	95.1	109.5	104.7	103.1	108.4	103.4	101.9	103.7	93.0
Moderately-high	115.6	84.9	101.0	87.1	96.8	82.1	112.3	107.1	123.6	101.0	112.2	69.2
Super-high	125.8	93.4	98.6	101.2	99.0	28.1	132.9	124.3	154.6	124.5	127.5	23.0

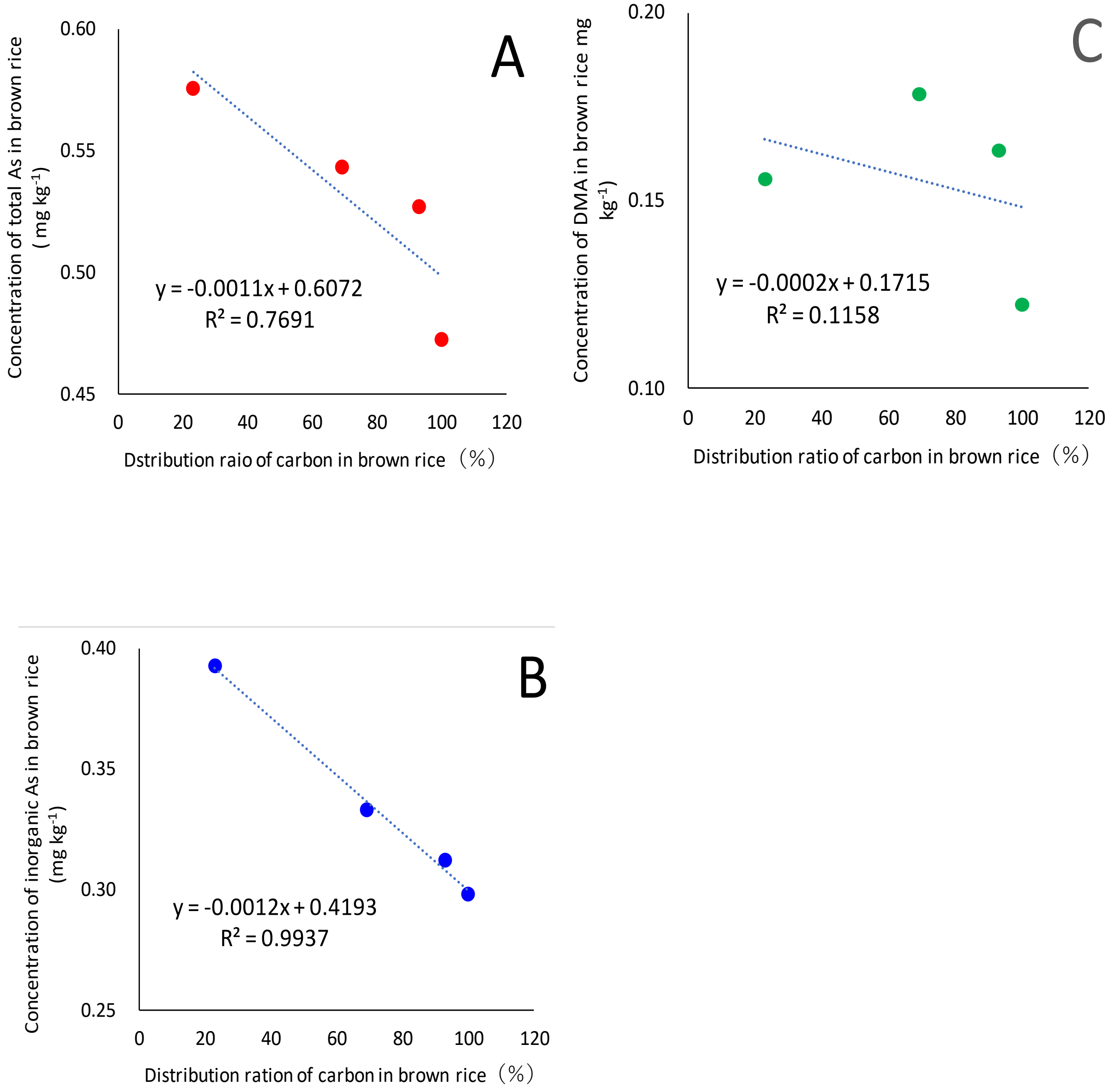


Figure 4.6. Relationship between the concentration of total As (A), inorganic As (B) and DMA (C) in brown rice and the distribution rate of carbon in brown rice.

Table 4.10. Effect of temperature and CFS application on the As/carbon ratio of the distribution ration in the parts of rice plant.

Temperature treatment	Under internode 3	Node 2	Internode 2	Node 1	Internode 1	Brown rice
Ambient	1.00	1.00	1.00	1.00	1.00	1.00
Mildly-haigh	0.92	0.94	0.88	0.93	1.06	1.12
Moderately-high	1.03	0.79	0.82	0.86	0.86	1.19
Super-high	0.95	0.75	0.64	0.81	0.78	1.22
Control	1.00	1.00	1.00	1.00	1.00	1.00
CFS15	1.11	0.67	0.96	0.75	1.09	0.81
CFS30	1.13	0.79	1.00	0.76	1.12	0.91

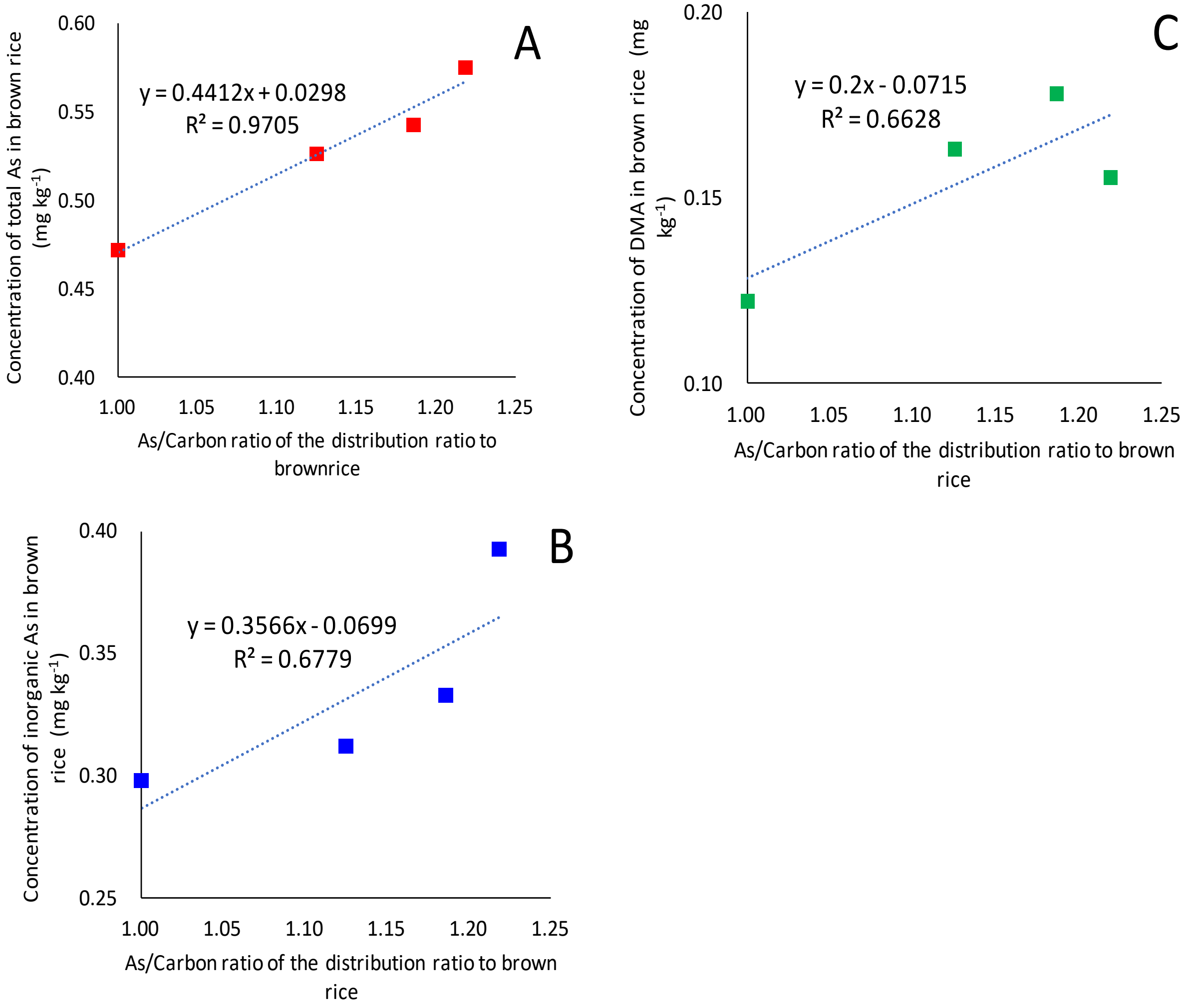


Figure 4.7. Relationship between the concentration of total As (A), inorganic As (B) and DMA (C) in brown rice and the As/Carbon ration of distribution ratio to brown rice.

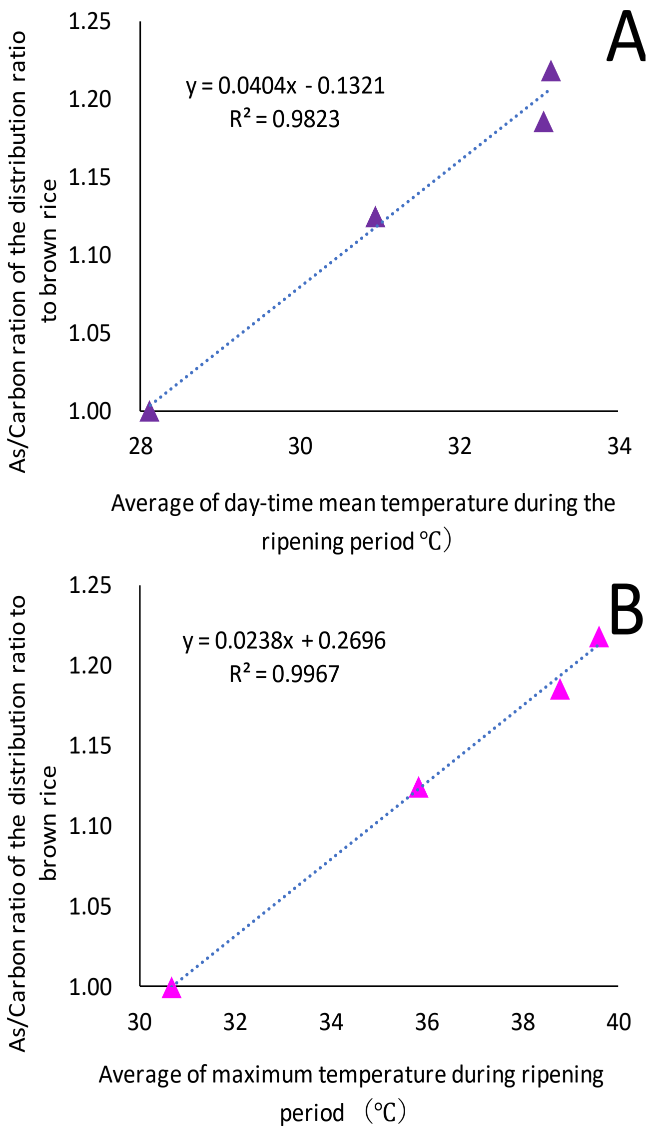


Figure 4.8. Relationship between As/Carbon ratio of the distribution ratio to brown rice and average of day-time mean temperature (A)and maximum temperature (B) during the ripening period.

Table 4.S1. Temperature effect on yield and yield components.

		Brown rice yeild (g pot^{-1})	Panicle number (pot^{-1})	Grain number (Pancle^{-1})	Culm length (cm)	Panicle length (cm)
Ambient	Control	19.6 ± 1.2	21.7 ± 0.7	54.6 ± 2.9	57.3 ± 0.8	15.4 ± 0.5
	CFS15	18.6 ± 1.1	19.3 ± 2.0	63.7 ± 4.9	49.1 ± 1.5	25.3 ± 1.4
	CFS30	22.4 ± 2.2	20.0 ± 2.6	70.3 ± 4.3	51.2 ± 1.1	25.3 ± 0.7
Mildly-haigh	Control	18.7 ± 0.9	21.7 ± 2.0	61.2 ± 2.9	58.4 ± 3.0	16.7 ± 1.0
	CFS15	21.3 ± 2.0	22.3 ± 0.9	63.1 ± 4.8	55.4 ± 3.8	23.4 ± 3.0
	CFS30	23.6 ± 0.5	21.0 ± 0.6	70.9 ± 1.4	57.9 ± 3.5	20.8 ± 2.4
Moderately-h	Control	14.1 ± 0.7	22.0 ± 1.7	62.7 ± 1.7	60.3 ± 1.0	15.8 ± 0.1
	FM15	11.7 ± 1.2	19.0 ± 1.5	67.6 ± 5.4	49.6 ± 2.8	24.9 ± 0.1
	FM30	16.5 ± 1.2	19.0 ± 2.6	68.4 ± 1.4	48.7 ± 2.6	25.9 ± 0.3
Super-high	Control	5.4 ± 1.0	22.7 ± 3.2	66.3 ± 3.2	63.2 ± 2.4	17.7 ± 0.5
	CFS15	3.4 ± 1.0	18.0 ± 2.1	63.6 ± 3.3	49.6 ± 1.8	25.0 ± 1.0
	CFS30	3.1 ± 0.7	19.7 ± 2.7	68.7 ± 4.3	50.8 ± 2.0	25.2 ± 0.4

Table 4.S2. Temperature effect on dry matter weight by parts in rice plant

Temperature treatment	Material application	Brown rice (g pot^{-1})	Internode 1 (g pot^{-1})	Node1 (g pot^{-1})	Internode 2 (g pot^{-1})	Node 2 (g pot^{-1})	Under node3 (g pot^{-1})	Sum of stem (g pot^{-1})
Ambient	Control	19.6 ± 1.2	6.6 ± 0.2	0.30 ± 0.02	6.4 ± 0.1	0.47 ± 0.04	7.3 ± 0.4	21.1 ± 0.7
	CFS15	18.6 ± 1.1	5.6 ± 0.3	0.35 ± 0.02	6.0 ± 0.3	0.55 ± 0.04	8.7 ± 0.8	21.2 ± 1.1
	CFS30	22.4 ± 2.2	6.0 ± 0.7	0.30 ± 0.05	5.8 ± 0.7	0.43 ± 0.08	9.6 ± 1.1	22.2 ± 2.8
Mildly-haigh	Control	18.7 ± 0.9	7.3 ± 0.2	0.33 ± 0.00	7.8 ± 0.1	0.57 ± 0.01	9.1 ± 0.2	25.1 ± 0.2
	CFS15	21.3 ± 2.0	6.2 ± 0.5	0.40 ± 0.03	6.7 ± 0.5	0.68 ± 0.06	10.7 ± 1.3	24.7 ± 2.3
	CFS30	23.6 ± 0.5	7.3 ± 0.2	0.36 ± 0.01	6.7 ± 0.2	0.54 ± 0.03	10.0 ± 1.1	25.0 ± 0.9
Moderately-high	Control	14.1 ± 0.7	6.5 ± 0.4	0.29 ± 0.02	7.4 ± 0.4	0.45 ± 0.08	11.2 ± 1.1	25.9 ± 0.7
	CFS15	11.7 ± 1.2	6.3 ± 0.3	0.30 ± 0.01	6.7 ± 0.3	0.49 ± 0.03	10.1 ± 0.9	23.9 ± 0.8
	CFS30	16.5 ± 1.2	6.1 ± 0.9	0.37 ± 0.09	6.1 ± 1.1	0.63 ± 0.17	10.0 ± 1.9	23.2 ± 4.0
Super-high	Control	5.4 ± 1.0	6.2 ± 0.5	0.30 ± 0.03	7.1 ± 0.6	0.54 ± 0.06	10.4 ± 1.1	24.5 ± 0.6
	CFS15	3.4 ± 1.0	6.0 ± 0.4	0.31 ± 0.04	6.1 ± 1.4	0.44 ± 0.03	12.9 ± 2.0	25.6 ± 1.9
	CFS30	3.1 ± 0.7	6.1 ± 0.5	0.39 ± 0.04	6.2 ± 1.0	0.54 ± 0.09	9.5 ± 1.1	22.8 ± 2.5

Table 4.S3. Concentration of As in brown rice

Temperature treatment	Material application	Inorganic As (m g kg^{-1})	DMA (m g kg^{-1})	Total As (m g kg^{-1})
Ambient	Control	0.313 ± 0.008	0.122 ± 0.023	0.490 ± 0.060
	CFS15	0.293 ± 0.007	0.112 ± 0.020	0.494 ± 0.033
	CFS30	0.288 ± 0.011	0.134 ± 0.035	0.432 ± 0.013
Mildly-haigh	Control	0.340 ± 0.007	0.216 ± 0.086	0.617 ± 0.070
	CFS15	0.307 ± 0.007	0.128 ± 0.023	0.497 ± 0.021
	CFS30	0.289 ± 0.004	0.147 ± 0.023	0.466 ± 0.029
Moderately-high	Control	0.344 ± 0.011	0.209 ± 0.023	0.609 ± 0.009
	CFS15	0.345 ± 0.010	0.172 ± 0.007	0.576 ± 0.013
	CFS30	0.311 ± 0.006	0.154 ± 0.013	0.444 ± 0.009
Super-high	Control	0.414 ± 0.005	0.157 ± 0.022	0.627 ± 0.008
	CFS15	0.394 ± 0.017	0.127 ± 0.017	0.530 ± 0.016
	CFS30	0.370 ± 0.022	0.183 ± 0.090	0.569 ± 0.043

Table 4.S4. Concentration of total As in the each parts of rice straw.

Temperature treatment	Material application	Internode 1 (mg kg^{-1})	Node1 (mg kg^{-1})	Internode 2 (mg kg^{-1})	Node 2 (g pot^{-1})	Under node3 (g pot^{-1})
Ambient	Control	5.9 ± 0.7	27.5 ± 3.2	4.2 ± 0.2	21.5 ± 0.8	3.6 ± 0.3
	CFS15	7.6 ± 0.4	21.2 ± 0.5	5.3 ± 0.3	16.7 ± 1.4	4.5 ± 0.5
	CFS30	5.7 ± 0.1	19.0 ± 1.9	4.2 ± 0.3	17.0 ± 0.9	2.9 ± 0.1
Mildly-haigh	Control	6.9 ± 0.7	26.7 ± 0.3	3.6 ± 0.3	20.8 ± 0.9	3.1 ± 0.4
	CFS15	8.3 ± 0.7	18.7 ± 0.8	4.9 ± 0.2	14.3 ± 1.3	3.7 ± 0.2
	CFS30	5.1 ± 0.3	17.2 ± 0.7	3.4 ± 0.2	15.1 ± 0.3	3.1 ± 0.4
Moderately-high	Control	5.0 ± 0.3	19.7 ± 0.7	3.4 ± 0.3	17.9 ± 2.7	3.4 ± 0.3
	CFS15	6.6 ± 0.9	22.1 ± 3.0	5.2 ± 0.8	13.4 ± 0.2	3.9 ± 0.2
	CFS30	5.4 ± 0.6	15.8 ± 4.1	3.3 ± 0.7	12.4 ± 2.1	2.7 ± 0.3
Super-high	Control	6.3 ± 0.6	23.7 ± 1.7	3.6 ± 0.3	17.7 ± 2.2	3.6 ± 0.2
	CFS15	6.0 ± 0.6	18.9 ± 3.2	3.9 ± 0.0	13.9 ± 2.1	2.9 ± 0.9
	CFS30	4.2 ± 1.0	14.7 ± 3.0	3.5 ± 0.6	10.9 ± 0.5	3.1 ± 0.6

Table 4.S5. Concentration of carbon in each parts of rice plants.

Temperature treatmen	Material application	Inter node 1 (g kg^{-1})	Node1 (g kg^{-1})	Inter node 2 (g kg^{-1})	Node 2 (g kg^{-1})	Under node3 (g kg^{-1})	Brown rice (g kg-1)
Ambient	Control	401.4 ± 3.6	375.0 ± 3.2	416.5 ± 3.90	391.3 ± 1.5	419.6 ± 5.29	416.4 ± 3.2
	CFS15	397.5 ± 2.8	368.4 ± 10.2	402.1 ± 4.79	390.5 ± 3.6	405.0 ± 5.27	405.2 ± 4.4
	CFS30	381.5 ± 1.1	361.9 ± 0.3	392.2 ± 6.24	395.6 ± 4.4	391.4 ± 2.03	418.1 ± 1.3
Mildly-haigh	Control	401.0 ± 6.7	375.6 ± 1.6	414.1 ± 5.51	389.8 ± 1.3	417.3 ± 5.18	417.5 ± 3.7
	CFS15	400.6 ± 6.4	372.8 ± 1.9	406.6 ± 10.02	388.4 ± 1.8	405.2 ± 4.38	408.9 ± 1.6
	CFS30	383.3 ± 8.1	379.9 ± 0.1	393.3 ± 3.08	388.9 ± 1.1	399.0 ± 1.85	418.5 ± 1.2
Moderately-high	Control	407.5 ± 4.4	369.8 ± 3.4	418.4 ± 4.47	387.4 ± 2.7	422.0 ± 2.09	415.3 ± 2.9
	CFS15	404.1 ± 5.8	376.7 ± 7.6	410.2 ± 7.79	391.6 ± 2.9	393.7 ± 5.91	414.8 ± 3.3
	CFS30	381.0 ± 1.1	369.9 ± 1.2	395.4 ± 3.30	390.7 ± 0.1	394.2 ± 2.92	408.7 ± 1.6
Super-high	Control	397.7 ± 2.0	368.5 ± 23.4	422.8 ± 5.48	385.8 ± 1.2	415.8 ± 7.97	407.4 ± 2.1
	CFS15	402.6 ± 2.2	341.8 ± 0.2	405.8 ± 9.89	388.4 ± 2.5	393.6 ± 1.70	411.9 ± 0.7
	CFS30	387.6 ± 0.0	382.7 ± 0.0	396.2 ± 0.00	394.3 ± 0.0	389.5 ± 0.00	409.3 ± 0.0

Table 4.S6. Accumulation of total As in each parts of rice plant.

Temperature treatment	Material application	Brown rice (μ g pot^{-1})	Internode 1 (μ g pot^{-1})	Node1 (μ g pot^{-1})	Inter node 2 (μ g pot^{-1})	Node 2 (μ g pot^{-1})	Under internode 3 (μ g pot^{-1})	Sum of straw (μ g pot^{-1})	SUM (μ g pot^{-1})
Ambient	Control	9.7 ± 1.6	38.5 ± 3.5	8.1 ± 0.5	27.0 ± 1.3	10.0 ± 0.6	26.3 ± 1.2	109.9 ± 4.6	119.5 ± 3.6
	CFS15	9.1 ± 0.4	42.4 ± 0.3	7.5 ± 0.5	31.8 ± 3.4	9.1 ± 0.2	39.7 ± 6.8	130.5 ± 9.3	139.7 ± 9.7
	CFS30	9.7 ± 1.1	33.7 ± 3.5	5.6 ± 0.6	23.8 ± 1.3	7.2 ± 1.2	28.0 ± 4.2	98.4 ± 10.5	108.1 ± 11.6
Mildly-haigh	Control	11.4 ± 0.9	49.6 ± 3.8	8.8 ± 0.1	28.4 ± 1.9	11.9 ± 0.5	28.1 ± 3.7	126.7 ± 7.8	138.2 ± 7.6
	CFS15	10.7 ± 1.4	52.6 ± 8.9	7.4 ± 0.7	32.8 ± 1.8	9.6 ± 0.2	40.2 ± 5.6	142.7 ± 16.3	153.4 ± 17.6
	CFS30	11.0 ± 0.9	37.2 ± 2.0	6.1 ± 0.2	22.7 ± 1.9	8.1 ± 0.3	31.3 ± 4.9	105.5 ± 7.8	116.5 ± 8.6
Moderately-high	Control	8.6 ± 0.5	32.6 ± 3.2	5.8 ± 0.7	25.4 ± 2.9	7.8 ± 0.9	39.0 ± 6.8	110.6 ± 9.4	119.3 ± 9.7
	CFS15	6.7 ± 0.5	42.0 ± 7.4	6.6 ± 1.0	34.8 ± 5.7	6.6 ± 0.5	39.3 ± 2.6	129.3 ± 9.4	136.0 ± 8.9
	CFS30	7.3 ± 0.5	32.5 ± 5.9	5.4 ± 1.1	20.3 ± 5.6	7.2 ± 1.2	26.8 ± 4.8	92.2 ± 18.2	99.5 ± 18.6
Super-high	Control	3.4 ± 0.7	38.4 ± 1.7	7.1 ± 0.2	24.8 ± 0.3	9.3 ± 0.3	37.8 ± 6.3	117.3 ± 5.9	120.7 ± 5.8
	CFS15	1.8 ± 0.6	35.3 ± 2.4	5.7 ± 0.6	23.8 ± 5.9	6.1 ± 1.0	34.9 ± 7.5	105.9 ± 6.4	107.7 ± 6.8
	CFS30	1.8 ± 0.5	25.5 ± 7.0	5.9 ± 1.7	22.6 ± 6.9	6.0 ± 1.3	30.7 ± 9.5	90.7 ± 23.1	92.5 ± 23.5

Table 4.S7. Accumulation of carbon in each parts of rice plant

Temperature treatment	Material application	Brown rice (g pot^{-1})	Internode 1 (g pot^{-1})	Node1 (g pot^{-1})	Internode 2 (g pot^{-1})	Node 2 (g pot^{-1})	Under internode 3 (g pot^{-1})	Sum of straw (g pot^{-1})	SUM (g pot^{-1})
Ambient	Control	6.9 ± 0.5	2.6 ± 0.0	0.11 ± 0.01	3.1 ± 0.2	0.18 ± 0.01	8.9 ± 0.4	14.8 ± 0.6	21.7 ± 1.0
	CFS15	6.4 ± 0.4	2.4 ± 0.1	0.13 ± 0.01	3.5 ± 0.3	0.22 ± 0.01	8.6 ± 0.3	14.7 ± 0.7	21.2 ± 1.1
	CFS30	8.0 ± 0.8	2.2 ± 0.3	0.11 ± 0.02	3.8 ± 0.4	0.17 ± 0.03	8.7 ± 1.0	14.9 ± 1.7	22.9 ± 2.5
Mildly-haigh	Control	6.6 ± 0.3	3.1 ± 0.0	0.12 ± 0.00	3.8 ± 0.1	0.22 ± 0.01	10.5 ± 0.1	17.7 ± 0.1	24.4 ± 0.4
	CFS15	7.4 ± 0.7	2.7 ± 0.2	0.15 ± 0.01	4.4 ± 0.6	0.27 ± 0.02	10.0 ± 1.0	17.5 ± 1.9	24.9 ± 2.4
	CFS30	8.4 ± 0.2	2.6 ± 0.1	0.14 ± 0.00	3.9 ± 0.4	0.21 ± 0.01	10.0 ± 0.4	16.8 ± 0.9	25.3 ± 1.0
Moderately-high	Control	5.0 ± 0.2	3.0 ± 0.2	0.11 ± 0.01	4.7 ± 0.5	0.17 ± 0.03	10.9 ± 0.3	18.9 ± 0.6	23.9 ± 0.8
	CFS15	4.1 ± 0.4	2.7 ± 0.2	0.11 ± 0.00	4.1 ± 0.4	0.19 ± 0.01	9.4 ± 0.3	16.6 ± 0.7	20.7 ± 1.1
	CFS30	5.7 ± 0.5	2.3 ± 0.4	0.14 ± 0.04	4.0 ± 0.8	0.25 ± 0.07	9.2 ± 1.7	15.9 ± 3.1	21.6 ± 3.5
Super-high	Control	1.9 ± 0.3	2.8 ± 0.2	0.11 ± 0.01	4.4 ± 0.4	0.21 ± 0.02	10.2 ± 0.3	17.7 ± 0.5	19.6 ± 0.1
	CFS15	1.2 ± 0.3	2.4 ± 0.6	0.11 ± 0.01	5.2 ± 0.8	0.17 ± 0.01	10.1 ± 0.8	18.0 ± 1.7	19.2 ± 1.4
	CFS30	1.1 ± 0.2	2.4 ± 0.4	0.15 ± 0.02	3.8 ± 0.4	0.21 ± 0.04	8.9 ± 1.0	15.4 ± 1.8	16.5 ± 1.9

4. Discussion

4.1. Increase in arsenic concentration in brown rice due to high temperatures and the reduction effect of converted furnace slag application

Muehe et al. (2019) reported important information on the relationship between elevated temperatures and soil As content, and warned of possible future rice cultivation at elevated temperatures. They noted that an increase in the As concentration in rice grains with the increase in temperature could seriously affect humans. In the present study, the effect of temperature on the As concentration in paddy rice during the ripening period was investigated in a temperature-graded chamber. A significant positive correlation was observed between arsenic concentration in brown rice and temperature. The significance probability of the mean daily maximum temperature and the mean daytime temperature was higher than the mean daily temperature and the mean daily minimum temperature.

The Japanese Ministry of Agriculture, Forestry and Fisheries (2019) conducted a multiple regression analysis of 3007 rice sites across Japan produced from 2013-2016 and noted that temperature in the first 2-4 weeks after heading was a significant explanatory variable and its adjusted coefficient of determination was high, along with an available As concentration in the soil. In other words, it is assumed that temperature after heading is a crucial factor affecting the arsenic concentration in brown rice, and the present study confirmed the prediction of the Japanese Ministry of Agriculture, Forestry and Fisheries (2019).

In the present study, we clarified that the application of CFS significantly reduced the brown rice As concentration even in the high-temperature condition compared to the non-application control (Table 4.4). CFS is a useful soil conditioner for replenishing silicate and iron, and because it is relatively inexpensive, it is widely used in paddy rice cultivation in Japan. We have already reported that the application of CFS effectively reduces brown rice As concentration in paddy rice cultivated in the field under normal temperature conditions (Matsumoto et al., 2015; Makino et al., 2016). In those reports, we pointed out that the As reduction effect by the application of CFS is mainly due to the competitive suppression effect by silicate, not to the adsorption effect of iron. The application of CFS did not affect the concentration of As in the soil solution, but we observed an increase in the concentration of silicate. Therefore, the As adsorption effect of iron including in CFS was not large, and the reason for the reduction of As concentration in brown rice by CFS was considered the competitive suppression of As uptake by the increased silicate in the soil solution. In fact, arsenite is the main form of arsenic in soil solution, and many researchers have reported that arsenic uptake is suppressed by the increase of silicate, which is similar in chemical form of arsenite, in the soil solution (Bogdan and Schenk, 2008; Li et al., 2018; Wang, 2016). Such the effect of silicate brought about a reduction in brown rice arsenic concentration in the CFS application as compared with the control even under the high-temperature treatment conditions performed in this study. Furthermore, Kaneda et al. (2010) demonstrated in a pot experiment that the rapid increase in leaf temperature associated with high-temperature treatment was suppressed from the ripening stage by the application of calcium silicate, and it has been speculated that it inhibits the consumption of carbohydrates accumulated in the leaf blade, thereby suppressing the decrease in yield and improving the quality of brown rice. Therefore, it is considered that the application of CFS is effective fertilizer management as an As reduction and degradation of yield and quality measure even under increasing temperature during the ripening period.

4.2. Mechanism of increase in brown rice arsenic concentration due to high temperature

Weber et al. (2010) showed that when the soil temperature was set to 5 °C, 14 °C and 23 °C, the higher the soil temperature, the higher the microbial activity and the greater the elution of iron and As from the soil solid phase to the soil solution. Neumann et al. (2017) used an XRF imaging device to increase iron and As accumulation on the root surface of paddy rice by 91% and 52%, respectively, when the soil temperature rises from 26.1 °C to 30.5 °C. It was shown that an increase in soil temperature promotes the elution of iron and As from the solid phase. In a pot test of paddy rice using TGC, we also found that the rate was significantly higher in the group subjected to high-temperature treatment from 1 week after heading to harvest than in the control group (Dhar et al. In press). In other words, the increase in soil temperature with increasing temperature has a significant effect on microbial activity in the soil, increasing the activity of iron-reducing bacteria and the amount of As adsorbed on iron also released into the soil solution because the activity of iron-reducing bacteria increases and the amount of divalent iron released from the solid phase into the soil solution is increased (Neumann et al., 2017) and the temperature The increase in As uptake by rice plants was found to increase the risk of As uptake. However, Neumann et al. (2017), who conducted an experiment in which only the soil temperature was increased, showed no increase in As concentration in brown rice, which they attributed to a physiological mechanism that inhibits the translocation of As into brown rice in rice.

In recent years, the mechanism of As accumulation in brown rice has been elucidated. It has been shown that As dissolved in soil solution is absorbed by the silicate transporter (Lsi1) and transported to the above-ground part. It has also been shown that the main form of As, iAs, is transferred to brown rice through the sieve tubes, and that the As is isolated at the nodes by the vacuoles of the sieve companion cells, and that the As is fractionated in the vacuole by glutathione (GSH) and phytochelatin (PC), which are combined with glutathione (GSH) and phytochelatin (PC) to become non-toxic. In other words, isolation of iAs in the nodes is considered to be an essential and significant mechanism to inhibit As translocation to brown rice. In fact, the concentration of As in the nodes was reported to be several times higher than that of straw. In this study, the total As concentration in the node 1 and node 2 was 22.6 ppm and 18.4 ppm, respectively, compared to 6.4 ppm and 4.6 ppm in the internode 1 and internode 2 in the ambient plot, respectively, and the total As concentration in the node was 3.5 to 4 times higher than that in the internodes. While the carbon distribution ratio in the nodes is 0.5-1.1% (Figure 4.5A), the As distribution ratio in the nodes shown in Figure 4.4A is 5-7%, indicating that As tends to accumulate relatively more in the nodes. If the As exclusion mechanism in these nodes is damaged by high temperature, the translocation of As to brown rice may accelerate, but the effect of high temperature on the As exclusion mechanism in these nodes has not been investigated in detail.

In this study, total As concentration tended to decrease with the high temperature in both straw and nodes, and there was a significant negative linearity in the relationship between As concentration and temperature in node 1, internode 2, and node 2 (Table 4.4). In particular, node 2 was highly significant, and it was clear that As concentration in the nodes decreased with high temperature. However, since the dry matter weight of straw tended to increase with high-temperature treatment (Table 4.3), it may be considered that the decrease in these concentrations was merely a dilution effect due to the increased dry matter weight. Therefore, we calculated the total As accumulation in each part of the straw and found that high temperatures significantly reduced the As accumulation in node 2 (Table 4.7). The relationship between As accumulation and high temperature in node 1 was not significant at the 5% level, but the significance probability p was 0.061, suggesting that high temperature may also reduce the accumulation of As in node 1. These results suggest that high temperatures during the ripening period may affect the As exclusion mechanism in the nodes. Furthermore, as shown in Figure 4.4, the distribution ratio of As in node 1 and node 2 tends to decrease with high temperature. Therefore, it is necessary to investigate the damage to the exclusion mechanism of As in nodes by genetic and proteomic analysis.

4.3. Decrease in yield due to high temperature and dry matter weight by parts of rice plant

In this test, cultivation was carried out under the same weather conditions up to 1 week after heading, and high-temperature treatment was performed 1 week after heading. In other words, the difference in dry matter weight for each part is considered to be due to the difference in weather conditions after ripening. Among them, brown rice was most affected by high temperatures, and the correlation with the maximum temperature was especially significant (Table 4.3). On the other hand, in the straw, the part that showed significant linearity with the temperature was in under internode 3, and the dry matter weight of under internode 3 increased as the temperature increased. Generally, during the ripening period, carbohydrates are transferred to brown rice, and the rice matures, but under high-temperature conditions, the weight of brown rice decreases, and the weight of straw, especially the straw in the lower internodes, increases. These phenomena are interpreted as the suppression of translocation of carbohydrates to brown rice due to high temperature. Tashiro and Wardlaw (1991) reported that the grain weight of Japonica rice varieties clearly decreased when the average daily temperature exceeded 26 °C during the ripening period. Many researchers have investigated the effect of high temperature on rice growth and reported that high temperatures during the ripening period significantly affected yield and quality deterioration (Peng et al., 2004).

The main reason for the decrease in yield under high-temperature conditions is thought to be the shortened ripening period (Nagato and Ebata, 1965; Kim, 1983; Tashiro and Wardlaw, 1989). In general, the end of ripening is caused by loss of activity in the source and/or sink. That is, ripening ends when their activities are lost due to the aging of panicle and foliage. As reported by many researchers, it was considered that the senescence of panicle and foliage accelerated with increasing temperature, which may have led to the early completion of maturation and the early cessation of panicle weight increase.

It was clear from the results of this study that the yield decreased with increasing temperature in the ripening period, which was due to the suppression of carbohydrate translocation during the ripening period. In fact, both the amount of carbon accumulation and the carbon distribution ratio was lower in the high-temperature treatments (Table 4.8, Figure 4.5). Similarly, because iAs is transferred to brown rice through the sieve pipe as described above, the amount of As accumulated in brown rice and the distribution rate of As were examined compared to carbon.

4.4. Effect of high temperature to As/carbon distribution ratio each part of the rice

In this study, it was clear that the concentration of As in brown rice increased with increasing temperature (Table 4.4), but the As accumulation in brown rice decreased with increasing temperature, similar to carbon (Table 4.7). On the other hand, there was no difference in the concentration of carbon in the part of rice plant and no difference in temperature treatment (Table 4.6). Differences in the distribution ratio were observed with high temperatures. In other words, this difference in the distribution ratio indicates the degree of suppression of ripening due to high temperature, and the fact that the distribution ratio to brown rice is significantly reduced due to high temperature is clear from the high distribution rate in straw. This is because the transfer of carbon from straw is suppressed.

There is no difference in the concentration of each part in carbon, and a constant concentration is maintained, so it is considered that there is no special exclusion mechanism or accumulation mechanism for carbon translocation (Table 4.6). On the other hand, as with carbon, the amount of As accumulated in brown rice decreased in the high-temperature area due to early termination of ripening (Table 4.7), but unlike carbon, the concentration of As in brown rice increased as the temperature increased (Table 4.4). The effect of high temperature on As distribution ratio in straw was not as clear as that of carbon (Figure 4.4, 4.5), although it was noticeable that the distribution ratio of As to brown rice decreased with increasing temperature, as was the case with carbon. This suggests that the effect of high temperature on the transfer and accumulation of carbon and As in brown rice may be different.

Therefore, the indices for each temperature treatment area were calculated for each part, where the ambient treatment was set at 100. In this case, it is clear that both the distribution ratio of As and carbon to brown rice decreases with high temperatures (Table 4.9). However, the distribution ratio of carbon tended to increase with the high temperature in all parts of the straw. In contrast, As showed a similar trend to carbon in the straw of under internode 3, but there was no clear relationship with high temperatures in other parts. Carbon is a major component of brown rice, and it is not an exaggeration to say that its conversion is the key to the yield of brown rice. The negative correlation between the decrease in the carbon distribution ratio and the As concentration in brown rice was found to be significant (Figure 4.6). In other words, this suggests that carbon translocation flow has a significant effect on the concentration of As in brown rice, and the As concentration decreases when the carbon translocation flow is high and increases when the carbon translocation flow is low. The concentration of As increases at higher temperatures suggests that the ratio of inhibition of translocation due to high temperatures is different between As and carbon because the amount of As transferred to brown rice also decreases at higher temperatures. In other words, the higher the temperature, the less dilution of As by carbon, the higher the concentration of As in the brown rice.

Therefore, As/carbon ratio of the distribution ratio was calculated for each part of the rice, and no effect of high temperature was observed in the straw under internode 3, but this value tended to decrease in other parts of the straw, while it tended to increase in the brown rice (Table 4.10). In other words, translocation of carbon is more susceptible to damage at higher temperatures than that of As. Since there was a significant positive correlation between the As/carbon ratio of the distribution ratio and the concentration of As in brown rice (Figure 4.7), the decrease in the carbon translocation rate due to high temperature was considered to be the main reason for the increase in the concentration of As in brown rice. Besides, this value increases with increasing temperature, i.e., As/carbon ratio of the distribution ratio has a very strong correlation with temperature, suggesting that the difference between As and carbon in flow rate widens with increasing temperature and that the translocation of carbon to brown rice is more susceptible to damage due to high temperature. Therefore, the concentration of As in brown rice under high temperature condition is considered to be high due to insufficient dilution by carbon. The significant difference between carbon and As translocation is that there is no difference in the concentration of carbon and no exclusion or accumulation mechanism in the translocation. If this sequestration mechanism is in any way affected by high temperatures, the transfer of As into the brown rice could be accelerated. On the other hand, the final amount of As accumulation was smaller at higher temperatures compared to the ambient condition. This may be due to the shortened ripening period caused by high temperatures. Therefore, it would then be necessary to clarify the rate of translocation of As with considering the mechanism of As exclusion in relation to the temperature over the ripening period. Furthermore, breeding rice varieties that are resistant to high-temperature ripening is effective in reducing As concentration in rice because the dilution effect by carbon may reduce the concentration of As.

Chapter 5

Conclusions and Recommendations

This chapter summarizes major conclusions based on the results of this study and gives a clear conception, which we expected. In this study, some newly important issues have been addressed. Further studies are also needed while made for recommendation purposes.

1. Conclusions

From this study summarized major conclusions are as following:

- Increased high temperature could be responsible to increase iAs concentration in brown rice grain during the ripening period. Due to the increased air temperature after heading, soil temperature also increased which followed to great impact through increasing As concentration in brown rice grain.

- Increased air temperature may increase adjacent soil temperature. When daily air temperature over 26 °C, adjacent soil temperature also increases rapidly to increase As in soil solution.

- After heading, 1-4 weeks have also been addressed in critical time to increase As concentration in soil solution when the daily temperature increases. The total As concentrations increased until 4th weeks up to 60.5 μg L^{-1} in the soil solution after heading, whereas from 5th weeks As concentration (55.0 μg L^{-1}) decreased during the ripening period.

- High temperature may increases arsenic accumulation in rice grain although rice cultivated in uncontaminated soil. The total As concentration in soil solution is particularly high for high-temperature treatment (56.0 μg L^{-1}) compare to control (49.1 μg L^{-1}) where rice cultivated in uncontaminated soil.

- Excessive As may accumulates in brown rice grain in day time compare to nighttime temperature. In the daytime, the highest temperature was detected in the range of 5.0 °C above the ambient temperature and was 3.5 °C times higher temperature compare to nighttime temperature.

- Significantly, a positive correlation was detected between brown rice yield and the air and soil temperatures, and the increase in air and soil temperatures resulted in a decrease in the yield at approximately 39% lower than the ambient treatment. The level of arsenic in straw, panicle, node, and grain of the rice plant samples collected from high temperatures are significantly higher compared to ambient treatment. those found in the samples collected from the arsenic affected areas. Since grain is used as daily feed for humans, arsenic exposure of human grain through rice and its impact should be carefully evaluated.

- In some cases, the thousand-grain weight was increased in the high-temperature conditions when the amount of carbohydrate would be preferentially accumulated in the rice grains.

- Arsenic and As species (iAs and DMA) also significantly increased with the increase of total arsenic when the high temperature increases. The proportions of the total As concentration was average 94% while iAs and DMA to total concentration were 73.7% and 20%, respectively in brown rice grain during the ripening period.

- Application of Si materials may promotable technique to reduce As concentration in rice grain under high-temperature conditions. Because Calcium silicate materials were significantly reduced As and As species in all high-temperature treatments after heading during the ripening period.

- Application of Fe materials was effective to reduce As and As species concentration in rice grain under high-temperature conditions. Two levels of CFS slag (CFS15 and CFS30) were significantly reduced As concentration in biomass, node, and grain even in high-temperature conditions which indicated to increase grain yield.

- The application of CFS is effective to reduce the As concentration in all parts of rice plant even in maximum and the mean temperature were high.

- CFS application suppressed absorption of As because each parts of rice plant As accumulation significantly reduced when maximum temperature verified as a covariate.

- Node associated to accumulate more As compare to straw in the analysis of each parts of rice straws even in high temperature conditions. Moreover, in the present study, we detected that the total As concentration in the node was 3.5 to 4 times higher than internode through the relationship between carbon (0.5-1.1%) and As (5-7%) distribution ratio in the nodes.

- A larger difference was found between As and carbon distribution ration to brown rice with the day time mean and maximum temperature. When As a decrease carbon distribution and accumulation are more than a decrease in the As distribution rate with the high temperature, then ultimately may affect to brown rice yield which we examined in our present study for confirming.

- Total Arsenic concentration in edible parts of straws and nodes have been found to be relatively significant negative linearity in the relationship between As concentration and temperature in node 1, internode 2, and specifically node 2. Among the analysis of the total As concentration in nodes and straw of this studied, relatively higher levels of arsenic reduced in node 2 while influenced high temperature also appear to have significantly higher tendency to reduce accumulate arsenic in nodes of the rice plants after heading during the ripening period.

- The concentration of As in brown rice increased while high temperature increases, although As and carbon accumulation decreased in brown rice due to ripening period interrupted as longer for maturity grain with the high temperature.

- The present study found that there was different transfer and accumulation for carbon and As accumulation in brown rice under high temperature because of As translocation inhibited to accumulate more As in brown rice due to the insufficient carbon dilution during the ripening period which retarded to increase As concentration in brown rice under high temperature conditions.

2. Recommendations

It should be recommended noted that only the experiment be conducted in the paddy field, which is needed for estimating As toxicity. Some recent studies suggest that a significant portion of arsenic in paddy field differ exist as As toxicity. However, the present study, conducted in Wagner pots due to analytical limitations. Studies are needed to better understand the levels of arsenic toxicity in paddy fields.

- In this study variation in arsenic uptake among different paddy, varieties were not assessed. Japanese popular cultivar of "Koshihikari" was the predominant rice variety in this study. More studies are needed to better understand the uptake of arsenic by different resistant varieties of paddy, including the indica varieties for comparing under high-temperature conditions.

- From the results of pot experiments, it appears that during the ripening period As concentration increased in rice grain with the air and soil temperature increases. Appropriate field studies need to be carried out to better understand for measuring the levels of soil depth as activated to increase the high temperature in the soil solution and these processes and their relative importance in regulating arsenic contents of soils.

- Adsorption-desorption depends on a wide range of factors, including soil characteristics (e.g." sand, silt and clay contents), pH, Eh of both soil and water although those factors did not evaluate in our studies. The effect of other relevant factors on arsenic adsorption-desorption in agricultural soil should be carefully studied.

- In order to elucidate the mechanisms underlying the increase in the As concentration in rice grains due to high temperature, it is necessary to determine the effect of high temperature on the mechanisms of As accumulation and defense in rice plants from growth period to harvest in rice plant.

- Further investigations of agronomic methods are required in field conditions to determine all of the precise effects of increased temperatures.

- For the better understanding of total As concentration in node of As exclusion mechanism did not discussed in details for reducing As concentration in node 2 with the high temperature during the ripening period. Therefore, it is necessary to evaluate the exclusion mechanism of As in node by the analysis of genetically and proteomic.

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Summary

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.

摘要

ヒ素は環境中に比較的低濃度で存在する半金属元素である．農耕地土壌におけるヒ素の可溶性は土壌の酸化還元電位に強く影響され，酸化状態では五価のヒ酸として存在する割合が大きく，土壌中の鉄と結合されて液相に溶出され難い．一方，還元状態では三価の亜ヒ酸に変化し，二価鉄の溶出に伴って液相に溶出されるようになる．そのため，栽培期間の多くを湛水状態で栽培されるコメは他の穀物よりもヒ素濃度が高いことが知られている．近年，国際的な食品の規格等を策定するコーデックス委員会で，玄米無機ヒ素濃度の国際基準値が 0.35 mg/kg に設定され（2016 年），非汚染土壌においても玄米ヒ素濃度の低減が厳しく求められるようになっている．一方，IPCC 第 5 次評価報告書では将来的な高温傾向の継続が指摘されており，水稲栽培においては登熟期の高温による収量およびコメの品質への影響が懸念されている．このような背景をもとに，近年，荒尾ら（2018）により，茨城県つくば市の同一水田で出穂前後に湛水管理したコメ（1986-2014 年産）の無機ヒ素濃度は，出穂後の気温と有意な相関があり，出穂後の高温によりコメ中の無機ヒ素濃度が上昇することが示唆された．また，農林水産省（2019）が全国 3007 か所で生産されたコメを調査したところ，出穂後 2 週間～4 週間の栽培地点の日平均気温とコメ中の無機ヒ素濃度は正の相関関係を示すことが明らかとなった．そこで，本研究では，温度勾配型チャンバー（TGC）を使用して，登熟期間中の温度が土壌中のヒ素の溶出に及ぼす影響および玄米中のヒ素濃度に及ぼす影響を検証することとした．また，これまでにヒ素濃度低減のために提案されている種々の土壌改良資材の高温条件下での有効性を評価した．さらに，部位別のヒ素および炭素濃度ならびにそれらの蓄積量を比較検討することにより，登熟期の高温が玄米ヒ素濃度を上昇させるメカニズムを明らかにすることを試みた．

登熟期の高温が玄米ヒ素濃度を上昇させるかどうかを検証するために，ヒ素非汚染の灰色低地土を充てんしたワグネルポット（1/5000a）で栽培されたイネ‘コシヒカリ’を出穂 1 週間後に 3 基の TGC に搬入し，収穫まで栽培した．各 TGC は入り口から奥にかけてスリット入りビニルで 3 分割され，入り口から最初の区画を対照区とし，真ん中の区画を高温処理 1 区，奥側の区画を高温処理 2 区とした．対照区と 2 つの高温処理区の気温と地温には明瞭な温度差が観察されたが，2 つの高温処理区の間の気温と地温に差異は大きな差異は観察されなかった．そのため，両高温処理区を高温処理区として一つにまとめて以後の解析を行った．登熟期間中の土壌溶液中の総ヒ素濃度は、高温処理区の方が対照区より有意に高く推移した．すなわち，高温により土壌中のヒ素の溶出が促進されると考えられた．高温処理区の玄米収量は、登熟歩合の低下によって対照と比較して約 17%減少した．玄米中の有機態ヒ素（ジメチルアルシン酸（DMA）およびモノメチルアルソン酸（MMA））の濃度は処理間に差異は認められなかった．これに対し，毒

性の高い無機 As の濃度は、高温処理の方が対照よりも高くなる傾向を示し(p = 0.065)，登熟期の高温が玄米ヒ素濃度を上昇させる可能性を示唆した.

ケイ酸資材（ケイ酸カルシウム）の施用が出穂期の高温条件下で玄米ヒ素濃度の低減に有効であるかを検証した．灰色低地土を充てんしたワグネルポット（1/5000a）にケイ酸カルシウムを 0，15ｇ施用して栽培した‘コシヒカリ’を出穂 1 週間後に TGC に搬入して収穫まで栽培した．TGC では入口より奥に向けて温度処理を露地区，微高温区，中高温区，超高温区の 4 水準に設定した．TGC 搬入から収穫までの平均気温は露地区より TGC 内で２℃高い範囲で推移していた．玄米の収量と気温および土壌温度との間には有意な負の相関があり，気温と土壌温度の上昇は収量の低下をもたらした．一方で，この玄米収量の低下はケイ酸カルシウムを施用することで有意に緩和された．玄米中のヒ素濃度は，気温および土壌温度と有意な正の相関関係を示し，気温と土壌温度の上昇に伴ってヒ素濃度が上昇した．また，ケイ酸カルシウムを施用した場合，無施用区に比べて，すべての温度範囲で玄米中のヒ素濃度が有意に低下し，高温条件下でもヒ素濃度を低下させる効果が認められた．以上の結果は，高温条件下でも玄米の収量低下やヒ素濃度の上昇をケイ酸塩資材の施用で緩和できる可能性を示唆している.

製鋼スラグの施用が出穂期の高温条件下で玄米ヒ素濃度の低減に有効であるかを検証した．灰色低地土を充てんしたワグネルポット（1/5000a）に製鋼スラグ 0，15，30ｇ施用して栽培したコシヒカリを出穂 1 週間後に TGC に搬入して収穫まで栽培した．TGC での温度設定はケイカル施用試験と同様に行った．収穫時には収量調査を行うとともに，高温による玄米ヒ素濃度のメカニズムを転流との関係から解析するために，イネを部位別（玄米，第 1 節間，第 1 節，第 2 節間，第 2 節，第 3 節間以下）に分けて分析を行った．玄米については無機ヒ素，DMA，総ヒ素および全炭素濃度を測定するとともに，それぞれの蓄積量を算出した．また，ワラではそれぞれの乾物重を測定するとともに，総ヒ素濃度と全炭素濃度を測定し，部位別の総ヒ素および全炭素蓄積量を算出した．出穂 1 週後から収穫までの日中気温および最高気温の平均気温は露地区に対してそれぞれ，微高温区で 2.8℃，5.2℃，中高温区で 4.9℃，8.1℃，超高温区で 5.0℃，8.9℃高い設定が得られた．高温処理期間中の日中平均気温を共変量として共分散分析を行ったところ，無機ヒ素濃度および総ヒ素濃度ともに有意な回帰の直線性が認められ，高温により玄米ヒ素濃度が上昇することが明らかとなった．また，製鋼スラグ 30g/ポット（1Mg/10a 相当）施用により玄米ヒ素濃度が有意に低下し，高温条件でも有効な玄米ヒ素濃度低減対策となりうることが示唆された.

無機ヒ素は篩管を通じて玄米へ流入することが明らかとなっていることから，炭素の動態と比較することにより，高温による玄米ヒ素濃度上昇の要因を検討した．節における炭素の分配割合が 0.5-1.1％であるのに対し，ヒ素の分配割合は 5～7％であり，ヒ素

は節に比較的多く蓄積されることが示された．もし，これらの節におけるヒ素の排除機構が高温によりダメージを受ければ，玄米への転流が高温により促進する可能性がある．ワラの各部位における総ヒ素蓄積量を算出したところ，第 2 節ではヒ素の蓄積量においても高温により有意に低下した．また，第 1 節のヒ素濃度と高温の関係は 5%水準では有意とは言えないが，有意確率 p は 0.061 となっており，高温による低下がある程度認められると推察された．すなわち，登熟期の高温は節におけるヒ素排除機構に影響を及ぼす可能性が示唆された．

ヒ素と炭素の転流割合のヒ素／炭素比を部位別に算出したところ，第 3 節以下のワラには高温による影響は認められないが，他のワラの部位ではこの値が減少する傾向が認められ，玄米では上昇する傾向が認められた．すなわち，これらの結果は高温になるほどヒ素に比べて炭素はワラから玄米へ移行しづらいことを意味している．言い換えれば，ヒ素よりも玄米への移行速度の低下が大きく，より高温のダメージを受けやすいと考えられる．そのため，玄米における分配率のヒ素／炭素比と玄米ヒ素濃度には有意な正の相関関係が認められることから，高温により炭素の転流割合が低下し，炭素による希釈効果が弱まることが登熟期の高温による玄米ヒ素濃度上昇の主要な要因と考えられた．

This thesis is based on the work presented in the following publications;

Protima Dhar, Kazuhiro Kobayashi, Kazuhiro Ujiie, Fumihiko Adachi, Junko Kasuga, Ikuko Akahane, Tomohito Arao and Shingo Matsumoto. Effect of High Temperature During the Ripening Period on the Arsenic Accumulation in Rice Grain Grown on Uncontaminated Soil with Relatively Low Level of Arsenic. Journal of Japanese Society of Agricultural Technology Management, Vol.27, No.4, 2021 (in press) (Chapter 2)

Protima Dhar, Kazuhiro Kobayashi, Kazuhiro Ujiie, Fumihiko Adachi, Junko Kasuga, Ikuko Akahane, Tomohito Arao and Shingo Matsumoto. The Increase in the Arsenic Concentration in Brown Rice Due to High Temperature During the Ripening Period and Its Reduction by Silicate Material Treatment. Agriculture Vol.10, 289, 2020, doi:10.3390/agriculture10070289. (Chapter 3)