



Screen for low-arsenic-risk rice varieties based on environment–genotype interactions by using GGE analysis

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Received: 4 October 2023 / Accepted: 15 November 2023
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Abstract Arsenic (As) accumulation in rice is a global health concern that has received increased attention in recent years. In this study, 12 rice genotypes were cultivated at four As-contaminated paddy sites in Taiwan. According to the different crop seasons and As levels in the soil, the sites were further divided into 18 environmental conditions. For As in soils, results showed that 67% of the studied environments were likely to represent As contamination. For As in rice, the mean total As concentration in brown rice grains ranged from 0.17 to 0.45 mg kg⁻¹. The analysis of variance for the environment effect indicated that grain As concentration was mainly affected by the environmental conditions, suggesting that there was a remarkable degree of variation across the trial environments. According to the combination

of the GGE biplot and cumulative distribution function of order statistics (CDFOS) analysis, five genotypes—TCS17, TCS10, TT30, KH139, and TC192—were regarded as stable, low-risk genotypes because the probability of grain As concentration exceeding the maximum permissible concentration (MPC) was lower for these genotypes across all environmental conditions. Particularly, TCS17 was recommended to be the safest rice genotype. Thus, grain As levels in the selected genotypes were applied to assess the health risk to Taiwanese residents associated with As exposure through rice consumption. Results showed that the upper 75th percentile values of the hazard quotient were all less than unity. This suggested that the health risk associated with consuming the selected rice genotypes was acceptable for most of the residents. The methodology developed here would be applicable to screen for stable, low-As-risk rice genotypes across multiple field environments in other regions or countries.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s10653-023-01795-2>.

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Keywords Arsenic · GGE biplot · Rank order statistics · Rice · Risk assessment

Introduction

Heavy metal pollution has triggered global concern because of its detrimental effects on the ecosystem and human health. Among the heavy metals, arsenic (As) is of particular concern due to its high toxicity

and ubiquitous spread in the environment (Chi et al., 2018). This metal can cause numerous deleterious health problems such as skin lesions and disorders of the respiratory, metabolic, and nervous systems (Juang et al., 2021; Sarwar et al., 2021). Furthermore, the inorganic forms of As (iAs)—arsenite (As (III)) and arsenate (As (V))—are highly toxic and have been classified as Group I carcinogens by the International Agency for Research on Cancer (IARC) (Chi et al., 2018). Long-term exposure to high levels of iAs may lead to a variety of cancers including lung, skin, liver, bladder, and kidney cancer (Duan et al., 2017; Sarwar et al., 2021). It is reported that the hazardous and carcinogenic effects caused by As exposure have already affected more than two million people worldwide (Samal et al., 2021).

Rice (*Oryza sativa* L.) is one of the most frequently consumed cereals in the world. In rice paddy fields, long-term flooding and poor soil conditions enhance As mobility and favor the transformation of As (V) to its more toxic form, As (III) (Juang et al., 2021; Syu et al., 2014). Therefore, rice is generally regarded as a typical As-accumulating crop plant. Arsenic exposure via rice consumption has become a serious problem and received increased attention, especially in South and South-East Asia, where rice is the major staple food (Li et al., 2011; Majumder & Banik, 2019). In the concerns of public health and food safety, it is imperative to develop effective approaches to better assess and manage the health risks of As associated with rice consumption.

It has been observed that the accumulation of As in grains differs between rice genotypes. Generally, hybrid rice cultivars tend to accumulate higher levels of As than japonica and indica cultivars (Gao et al., 2021; Rahman et al., 2007). In our previous study, significant differences in As levels in grains were found among 12 rice genotypes (Juang et al., 2021). Thus, a number of studies have been conducted to mitigate the health risk of dietary As exposure by screening for low-As-accumulating rice genotypes (Cao et al., 2014; Chi et al., 2018; Duan et al., 2017; Norton et al., 2012).

In addition to rice genotypes, environmental conditions such as the planting site and crop season are also key factors controlling As accumulation in grains. One of the most popular methods to evaluate the effects of genotype (*G*), environment (*E*), and genotype–environment interactions (*G*×*E*) on

metals accumulation is the genotype plus genotype-by-environment (GGE) biplot analysis (Cao et al., 2014; Chi et al., 2018; Duan et al., 2017). This analytical methodology was originally developed to analyze multiple environment trial data (Yan, 2001; Yan & Tinker, 2006). For example, Khan et al. (2021) employed the GGE biplot in combination with the additive main effect and multiplicative interaction model to analyze the yield and stability of Bambara groundnut in Malaysia. Moreover, a number of studies have utilized the GGE biplot to study the effect of GGE on the accumulation of heavy metals in rice and to screen for and recommend stable metal-excluding cultivars for commercial cultivation (Cao et al., 2014; Chi et al., 2018). Duan et al. (2017) emphasized that one can identify locally adapted rice cultivars with low accumulation of Cd and As in grain, which being stable across multiple sites and seasons. However, these low-metal-accumulating cultivars may not be suitable to the growth conditions in other regions.

Generally, the environmental quality standard of metals for farmland soils is set to protect agricultural production and human health (Gao et al., 2021). Due to large genotypic and environmental variations (Murugaiyan et al., 2021), however, rice planted in uncontaminated soils may accumulate higher As levels than the food safety standard (Juang et al., 2021, 2022). Therefore, for the prevention of consumer exposure to contaminated rice, it is practical to screen for stable, low-As accumulators based on the food safety standards of As in rice grains (Duan et al., 2017). The probability of grain As levels exceeding the maximum permissible concentration (MPC) is usually of interest for the protection of consumers' health. In this regard, the cumulative distribution function of order statistics (CDFOS) takes into account the deviation between rice As levels and the threshold, making it applicable to select stable, low-As-accumulating cultivars based on a conservative human health risk assessment (Juang et al., 2005).

Numerous studies have implemented GGE as a useful tool to identify stable and superior genotypes across multiple environment. However, the “low-As-accumulating” cultivars may not necessarily be the “low-As-risk” cultivars. The selection of stable, low-As-risk rice genotypes should be based on the combination of environment–genotype interaction, food quality standards, and health risk assessment. In the present study, the GGE biplot combined with

a CDFOS approach could provide a promising methodology for the screening of low-risk rice cultivars associated with dietary As exposure. The aims of this study were to (1) identify stable, As-excluding rice genotypes that are suitable to local growth conditions; (2) recommend low-As-risk rice cultivars by a combination of the GGE biplot and the CDFOS model; and (3) perform a human health risk assessment of As associated with the selected rice genotypes.

Materials and methods

Sampling sites and rice genotypes

Four rice paddy fields located in different regions in Taiwan—Dongshan in Ilan County, Beitou in Taipei City, Dacun in Changhua County, and Minsyong in Chiayi County—were selected as sampling sites in this study. These sites were all double-crop rice paddies and suffered from As contamination to different degrees. Prior to the field experiments, soil samples were collected from each site, and the basic properties and total As concentrations of the samples were investigated. According to the different crop seasons and As levels in the soil, the sites were further divided into 18 environmental conditions—DC10IL, DC10IM, DC10IH, DC09IIL, DC09IIM, DC09IIH, DS09IL, DS09IH, BT07II, BT07I, MS10IL, MS10IM, MS10IH, MS09IIL, MS09IIM, MS09IIH, MS07II, and MS07I. The details for the environmental conditions combined with different experiment sites (i.e., Dacun, Dongshan, Beitou, and Minsyong), soil As levels (i.e., low ($<30 \text{ mg kg}^{-1}$), medium ($30\text{--}60 \text{ mg kg}^{-1}$), and high ($>60 \text{ mg kg}^{-1}$)), cropping year (2018, 2020, and 2021), and growth seasons (spring and autumn crops) were given in Table S1.

Twelve rice cultivars that are widely grown in Taiwan—TK2, TK9, TK14, TK16, TNG71, TC192, TN11, KH139, TT30, TCS10, TCS17, and TCSW2—were selected for this study. Table S2 shows the agronomic characteristics in detail for the selected rice genotypes (Lu & Lu, 2010). Briefly, TK2, TK9, TK14, TK16, TNG71, TC192, TN11, KH139, and TT30 are japonica rice, which is the major type of rice for staple consumption. TCS10 and TCS17 are indica rice, and TCSW2 is glutinous rice. Most of indica or glutinous rice is used for food processing. For rice cultivation, seeds for each cultivar were

soaked in deionized water at $\sim 25 \text{ }^\circ\text{C}$ for a 36 h germination period in the dark. Then, the germinated seeds were sowed in nursery beds. After three weeks of sowing, rice seedlings were transplanted into the paddy sites. All of the cultivars were planted at all four sites from 2018 to 2021. During the experimental period, the irrigation frequency was set as around two times per week; sometimes, rainfall occurred and irrigation was reduced. In addition, the fertilizer application rates for total nitrogen, phosphorus, and potassium were 150 kg N ha^{-1} , $40 \text{ kg P}_2\text{O}_5 \text{ ha}^{-1}$, and $90 \text{ kg K}_2\text{O ha}^{-1}$, respectively. At maturity, unhusked rice grains were harvested from select plots of the study sites. The plots were arranged in a randomized complete block design with three replicates, and each plot contained at least three rice samples. Unhusked rice grains from the same plot were pooled together, placed in a nylon bag, and then transported to the lab for further analysis.

Determination of total As in soils and rice grains

Soil samples were air dried and ground to pass through a 2-mm sieve. The total As content of tested soils was determined by a modified nitric acid-hydrogen peroxide ($\text{HNO}_3/\text{H}_2\text{O}_2$) digestion (Liao et al., 2021; Meharg & Rahman, 2003). Each sample was further finely ground to pass through a 100 mesh sieve. Next, 0.1 g of finely ground soil was weighed into a digestion tube and 10 mL of 30% hydrogen peroxide was added. The temperature was gradually raised to $125 \text{ }^\circ\text{C}$ to digest for 2 h. After cooling for 0.5 h, 1 mL of 30% hydrogen peroxide was added into the tube and the temperature was raised to $125 \text{ }^\circ\text{C}$ for 0.5 h. Then, the digest was diluted in deionized water and made up to 50 ml, and then filtered through Whatman 42 filter paper. Finally, total As concentrations in the filtrates were determined by inductively coupled plasma mass spectrometer (ICP-MS/Agilent 7700 \times).

Rice grain samples were rinsed thoroughly with distilled water and oven dried. Samples were then dehusked and ground into a fine powder. According to the extraction of As by Huang et al. (2010), one used 10 mL of 0.28 M HNO_3 at $95 \text{ }^\circ\text{C}$ for 90 min to recover As from 0.1 to 0.2 g of each finely ground sample. After cooling, total As concentration in each extract was determined by ICP-MS, followed by a volume make up to 10 mL with deionized water.

The ICP-MS system was equipped with a standard glass concentric nebulizer and quartz double-pass spray chamber. Optimum operating parameters including the RF power, carrier gas flow rate, dilution gas flow rate, sample depth, extract 1, extract 2, cell entrance, cell exit, and cell gas flow were set according to our previous study (Juang et al., 2021). The As standard stock solution for ICP-MS (i.e., 1 mg As L⁻¹ in 2–3% nitric acid) was purchased from the Sigma-Aldrich Co. Ltd. and used to generate a five-point calibration curve (0.0, 2.0, 5.0, 10, and 20 µg L⁻¹) for quantification of As analysis. For quality assurance and control (QA/QC), the certified reference materials of soils (CRM 025–050) and rice flour (ERM BC211) were used to verify the recoveries of the total As concentrations in soils and rice samples, respectively (Liao et al., 2021; Syu et al., 2019, 2020). The mean recoveries of total As in soils and rice were 98% and 97%, respectively.

GGE biplot analysis

The multi-environment trial data of total As concentration in rice grains can be expressed as

$$Y_{ijk} = \mu + g_i + e_j + ge_{ij} + \varepsilon_{ijk} \quad (1)$$

where Y_{ijk} is the As concentration of genotype i in environment j and replicate k , μ is the grand mean, g_i is the main effect of genotype i , e_j is the main effect of environment j , ge_{ij} is the interaction effect of genotype i and environment j , and ε_{ijk} is the residual associated with genotype i in environment j and replicate k . First, the data should be checked using one-way analysis of variance (ANOVA). If the genotype–environment interaction is significant, then the data should be further analyzed using the GGE model. By decomposing and partitioning the singular values into the genotype and environment eigenvectors, Eq. (1) can be rewritten as

$$Y_{ijk} = \mu + e_j + (g_i + ge_{ij}) + \varepsilon_{ijk} = \mu + e_j + \sum_{n=1}^N \xi_{in} (\lambda_n \eta_{jn}) + \varepsilon_{ijk} \quad (2)$$

where λ_n is the square of the n th [$n=1$ to N ; $N=\min(g, e)$] singular value for the n th principal component, ξ_{in} is the eigenvector of genotype i for the n th singular vector, and η_{jn} is the eigenvector of environment j for the n th singular vector. Then, the first and second

principal components (PC1 and PC2) can be plotted directly to generate the GGE biplot (Atnaf et al., 2013; Khan et al., 2021; Yan et al., 2000; Tsai, 2022).

Cumulative distribution function of order statistics

To conservatively screen for a safe rice cultivar, the multi-environment trial data of total As concentration in rice grains (z_i) were transformed into order statistics by ranking them in ascending order, i.e., $z_1 \leq z_2 \leq z_3 \leq \dots \leq z_r \leq \dots \leq z_n$, where z_r is the r th smallest value of all the observation data, and n is the total sample size. Let z_k be the critical concentration (i.e., MPC) of As in rice grains, and $F(z_k)$ be the probability of an observation value smaller than z_k . Then, the r th CDF, $F_r(z_k)$, can be expressed as (Juang et al., 1998, 2005):

$$F_r(z_k) = \text{Prob}[z_i \leq z_k] = \sum_{i=r}^n \binom{n}{i} [F(z_k)]^i [1 - F(z_k)]^{n-i} \quad (3)$$

Human health risk assessment

For assessing human health risk associated with rice consumption, the guidance proposed by US EPA (US EPA, 1992; 2002) was adopted in this study. This guidance has been widely applied in characterizing the toxic effects caused by excessive heavy metal intake through food chain (Juang et al., 2021). The hazard identification of the present study was to estimate the health risk of iAs through the consumption of rice of Taiwan residents. In the exposure assessment, the iAs concentration distribution in selected rice cultivars was used as the input parameter. The estimated daily intake (EDI, mg kg⁻¹ d⁻¹) of local residents exposed to iAs associated with rice consumption was calculated as follows:

$$\text{EDI} = \frac{C_{\text{rice}} \times P_{\text{inorg}} \times P_{\text{white/brown}} \times \text{IR} \times \text{CF}}{\text{BW}} \quad (4)$$

where C_{rice} is the total As level in brown rice in the considered environment (mg kg⁻¹), P_{inorg} is the proportion of iAs to total As in brown rice, $P_{\text{white/brown}}$ is the proportion of iAs in white rice to brown rice, IR is the ingestion rate of white rice (g d⁻¹), CF is the conversion factor (0.001 kg g⁻¹), and BW is the body weight of the Taiwanese population (kg). In

the present study, P_{inorg} is set to be 0.75. This value is adopted from previous works which analyzed the published data of As in rice grains in Asian countries (Mandal et al., 2021, 2023). On the other hand, $P_{\text{white/brown}}$ depends mainly on the extent of rice polishing. Juang et al. (2022) considered the rice-polishing ratio in Taiwan and recommended a value of 0.65 for $P_{\text{white/brown}}$. Hence, $P_{\text{white/brown}}$ is set to be 0.65 in the present study. Moreover, because IR and BW in Eq. (4) are highly variable according to sex, the EDI and the corresponding health risks of female and male populations were estimated separately in the present study (Juang et al., 2022).

In dose–response assessment, the reference dose (RfD) of iAs should be determined. From US EPA IRIS (Integrated Risk Information System) database, the RfD was set to be $0.0003 \text{ mg kg}^{-1} \text{ d}^{-1}$ in this study. For risk characterization, the hazard quotient (HQ), representing the ratio of EDI to the RfD of iAs, was calculated as follows:

$$\text{HQ} = \frac{\text{EDI}}{\text{RfD}} \quad (5)$$

If the HQ exceeded unity, i.e., EDI exceeded RfD, then the potential hazardous effects could be a concern (Juang et al., 2022).

Statistical analyses

Descriptive statistics, including the arithmetic mean and standard deviation, were obtained using Microsoft Excel 2013. ANOVA and the GGE biplot were conducted in Genstat 20 (VSNi, Hertford, UK) following the method by Glaser (2012). In addition, Monte-Carlo simulation with 10,000 iterations was performed using the Oracle Crystal Ball software (Ver. 11.1.2.4.850, OracleR, CA, USA) to estimate EDI, HQ, and TR by considering the distribution of the independent variables (C_{rice} , IR, and BW) in Eq. (4).

Results and discussion

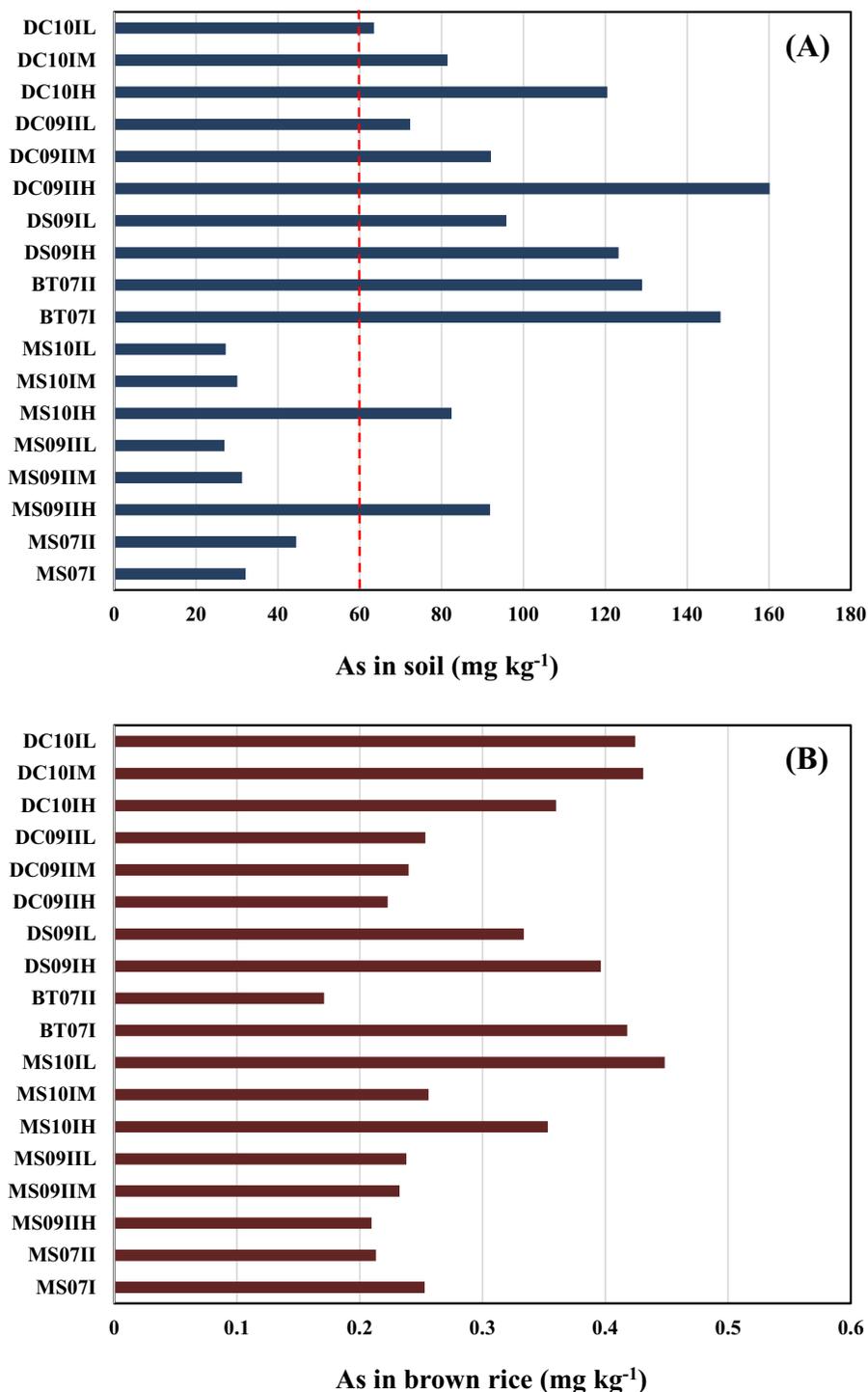
As concentration in soil and brown rice

There was a remarkable difference in soil As concentrations between the 18 environments (Fig. 1A). The mean soil As concentration ranged from

26.95 mg kg^{-1} in MS09IIL to $160.17 \text{ mg kg}^{-1}$ in DC09IIIH, with a 5.94-fold difference between the maximum and minimum levels. Twelve (67%) of the studied soils were likely to represent As contamination in terms of the maximum permissible standard (MPS) for As in agricultural land in Taiwan (i.e., 60 mg kg^{-1}) (Juang et al., 2021). Similarly, there was also a considerable difference in As levels in brown rice between environments (Fig. 1B). The total As concentration in brown rice ranged from 0.17 mg kg^{-1} in BT07II to 0.45 mg kg^{-1} in MS10IL, with a 2.65-fold difference, indicating a much lower difference than in the soil. Interestingly, even in highly contaminated soils with As contents higher than 120 mg kg^{-1} (i.e., DC09IIIH and BT07II), the grain As level might be low. In contrast, the highest mean As concentration in grains was observed in MS10IL, even though the soil As level was relatively low (27.26 mg kg^{-1}).

At the world level, Islam et al. (2016) found that the total As concentration in grains of rice ranged from 0.02 to 0.90 mg kg^{-1} . Chen et al. (2016) found the normal As concentration in brown rice was 0.216 mg kg^{-1} in Taiwan. In the present results, the mean total As concentration in rice grains ranged from 0.17 to 0.45 mg kg^{-1} , which was a little higher than the findings of Chen et al.'s (2016) study. It should be noted, however, that the rice in this study was grown in moderately or highly contaminated soils, with As levels ranging from 26.95 to $160.17 \text{ mg kg}^{-1}$. In addition, the absolute difference in grain As concentration was only 2.65-fold between the 18 environments, despite different As contamination levels. The relatively small variation in As accumulation in grains is consistent with our previous finding (Juang et al., 2022). At low As levels in the soil, a large proportion of As could be absorbed and translocated to grains because the biotic ligands within rice plants remained unsaturated; however, the absorption and translocation will be constrained because the biotic ligands within rice plants were nearly occupied and saturated at high As levels in the soil (Juang et al., 2022). Furthermore, previous studies have reported that the highest As concentration was found in rice roots, while the lowest was in grains (Islam et al., 2016; Juang et al., 2021). In addition, it is generally recognized that japonica rice has lower grain As concentrations than indica rice (Islam et al., 2016). Therefore, the relatively lower As levels

Fig. 1 **A** The concentration of total As in soils in the tested environments and **B** The concentration of mean total As in brown rice in the tested environments



in grains might also be due the present study using primarily japonica rice (9 out of 12 cultivars).

In order to set regulatory As standards for soils based on food safety considerations, many previous

studies were conducted to delineate the relationship between As concentrations in rice grains and in soils (e.g., Juang et al., 2022; Khan et al., 2010; Norton et al., 2013; Syu et al., 2015). Among these studies,

Khan et al. (2010) and Syu et al. (2015) reported that As concentrations in rice grains decreased with increasing soil As levels based on their pot experiments. In contrast, Norton et al. (2013) conducted pot experiments and found a positive correlation between As concentrations in soil and rice grains. In our recent field survey, however, As levels in rice grains were not significantly affected by soil As concentration (Juang et al., 2022). The inconsistency of these results may be due to the fact that As concentration in grains varies depending on factors such as the soil property, rice genotype, As bioavailability, and translocation (Syu et al., 2015). Furthermore, As accumulation in rice grains mainly depends on As bioavailability rather than total As in the soil (Ahmed et al., 2011). In a complicated field situation, therefore, it is difficult to predict the As levels in rice grains based solely on total As concentrations in the soil. One possible method to avoid this dilemma is to describe and analyze the experimental data in multiple environments using statistical methods such as the GGE biplot (Chi et al., 2018).

GGE biplot analyses

The ANOVA for environments indicated that grain As concentration was significantly affected by environment, genotype, and genotype–environment interactions (Table 1). The environment explained 73.80% of the variation, which was 11 times greater than the genotypic (6.28%) and five times greater than the genotype–environment interaction (12.87%) effects on the total variation. In this study, there was a remarkable degree of variation across the trial environments. In addition, a significant genotype–environment effect indicates the possible presence of different mega-environments with different winning genotypes (i.e., low As accumulators) (Atnaf et al., 2013).

Results of the GGE biplot analyses are shown in Fig. 2. PC1 and PC2 explained 81.41% of the

variability in As concentrations in brown rice. Figure 2A shows the mean vs. stability view of the GGE biplot. The single-arranged blue line represents the average environment coordination abscissa, which indicates a lower mean total As concentration in brown rice across the tested environments. The length of the vertical green line indicates the instability of the tested cultivars for As levels in brown rice (Yan, 2010; Yan et al., 2007). Based on the ranking biplot, the five genotypes with the lowest total mean As concentrations were TCS17, KH139, TCS10, TT30, and TC192. Among these five genotypes, KH139, TC192, and TT30 could be classified as stable, low-As accumulators because of their relative stability across all of the trial environments.

Figure 2B represents the “which-won-where” view of the GGE biplot. This plot helped to identify environmental-specific genotypes for As concentration. The five genotypes that were the furthest from the biplot center (i.e., TK2, TCS17, TCS10, TCSW2, and TK9) were linked with a polygon; thus, all genotypes were covered in the polygon. The five vertical lines running perpendicular to the polygon from the center divided the biplot into five different groups, three of which included at least one environment vector (Khan et al., 2021; Yan et al., 2007). According to the results of the analysis, the genotypes TK2, TCS17, and TCSW2 were found to be highly stable and the best performing (low As) genotype for each environmental group.

Figure 2C shows the discriminating power view of the GGE biplot. The length of the blue line in this biplot is proportional to the standard deviation of the genotype mean in the environment. Therefore, an environment with a longer blue line represented more discriminating power (i.e., the ability of an environment to distinguish genotype differences) toward the genotype in the environment (Atnaf et al., 2013; Yan et al., 2007). In the present results, MS10IL, DC10IM, DC10IH, BT07I, and DS09IH were the most discriminating environments

Table 1 Analysis of variance for grain As concentration of 12 rice cultivars grown at 18 environments of Taiwan

Sources of variation	DF	SS	MS	F value	P	%SS ^a
Environment (E)	17	5.43567	0.31975	279.50	<0.001	73.80
Genotype (G)	11	0.46243	0.04204	36.75	<0.001	6.28
G × E	187	0.94794	0.00507	4.43	<0.001	12.87
Residual	454	0.51936	0.00114			
Total	669	7.36540				

^aSum of squares (SS) of each effect by total SS

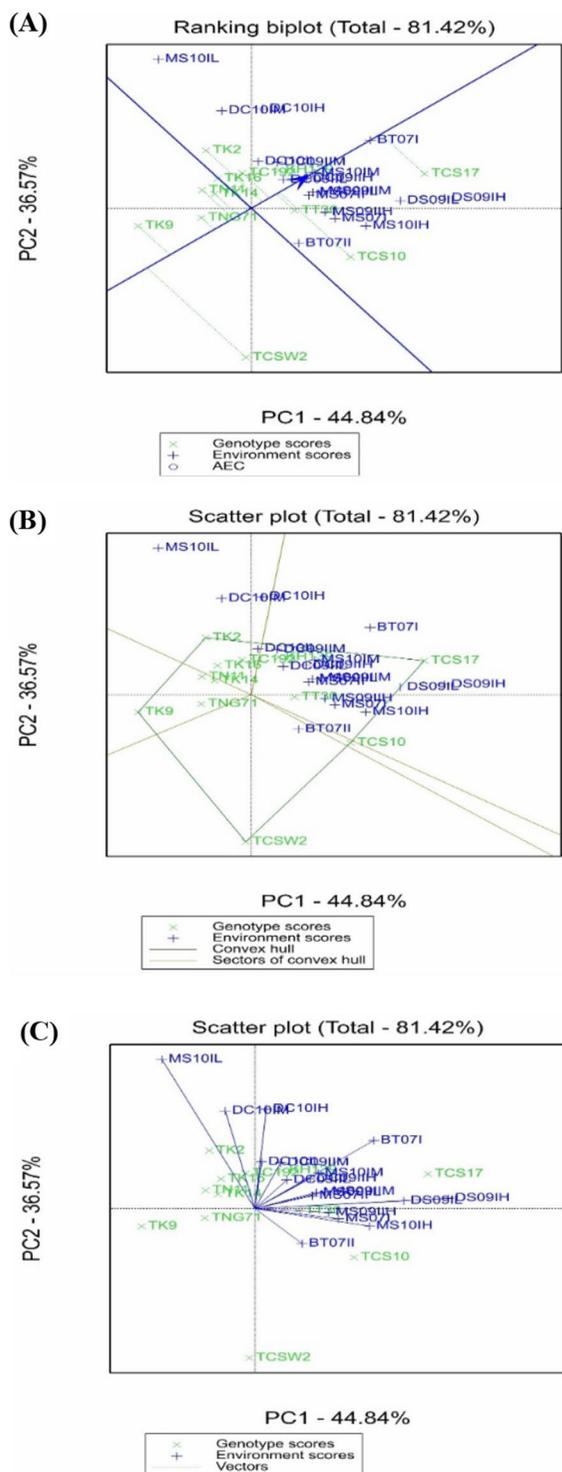


Fig. 2 The GGE biplots of As concentration in brown rice across all environmental trails to show **A** “Mean vs. Stability” view; **B** “Which-Won-Where” view; **C** “discriminating power” view. Green symbols are codes for rice genotypes. Blue symbols are codes for tested environments

toward the genotypes. In other words, these five environments could be regarded as the representative or ideal environments.

Rice consumption is generally regarded as the major source of dietary As exposure for populations whose staple food is rice (Duan et al., 2017). For food safety and public health, a number of strategies, including genotype selection, irrigation management, and fertilizer amendments, have been adopted to reduce As accumulation in rice grains (Islam et al., 2016; Juang et al., 2021; Roel et al., 2022). Some recent studies have investigated either genotypic or environmental effects on As accumulation in rice. For example, Syu et al. (2015) conducted pot experiments and investigated the difference in As levels in the grains of six rice genotypes commonly planted in Taiwan. Samal et al. (2021) studied the genotypic effects of As accumulation in 44 rice genotypes commonly grown in India. Numerous studies have investigated the geographical or environmental variation of As accumulation in rice grains in different countries, such as Bangladesh (Islam et al., 2017), China (Mu et al., 2019), Brazil (dos Santos et al., 2021), and Uruguay (Roel et al., 2022). Because As accumulation in rice grains has been reported to vary with genotype, environment, and genotype–environment interaction, some studies have employed GGE to analyze multi-environmental trial data to provide more comprehensive and reliable knowledge of low-As-accumulating cultivars (Chi et al., 2018; Duan et al., 2017). In the present results, a significant genotype–environment interaction was obtained, suggesting the adequacy of applying GGE for the analysis of experimental data. This result also indicated the possible presence of different mega-environments with different winning genotypes (Yan & Kang, 2002). Therefore, the rice genotypes with low As accumulation in grains might be different for different environments.

In the present results, the environment factor was predominant among all possible sources of variation, which is consistent with the findings proposed by Norton et al. (2009) and Ahmed et al. (2011). The large environment effect suggests that variation in rice grain As concentration was largely controlled by environmental factors rather than by genotype. The environment factor included the site, crop season, and As contamination in the soil. Some studies further discussed the effects of site and crop season on As accumulation by dividing

the source of variation in the environment factor (Ahmed et al., 2011; Chi et al., 2018). Based on the GGE analysis of multi-environment trials in southern China, Chi et al. (2018) proposed that crop season played an important role in the accumulation of As in rice grains. They further indicated that the autumn season was generally linked with reduced total As levels. Similar result was also observed in the present study. The mean total As level in brown rice was higher in the spring season (0.367 mg kg^{-1}) than that in the autumn season (0.223 mg kg^{-1}) across the studied sites (Fig. 1B). The season effect may be attributed to the difference of soil water contents caused by different rainfall amounts. In Taiwan, rainfalls in the spring seasons were usually higher than those in the autumn seasons, thus increased the dissolved As in soil solutions. The site effect was prominent in this study. The mean total As concentration in rice was 0.275, 0.294, 0.365, and 0.322 mg kg^{-1} at the MS, BT, DS, and DC site, respectively. This might be due to the difference of soil pH at different sites (Chi et al., 2018). On the other hand, the effect of As contamination in the soil was less obvious. Although more reliable attribute could be achieved by dividing the source of variation in the environment factor, the aim of the present study was mainly to screen for stable, low-As-accumulating rice cultivars across all field environments in Taiwan instead of delineating the detailed effects of different environment factors. Indeed, a number of rice genotypes with stable low accumulation of As in the grains were identified based on the multi-environmental trials. Because all the tested rice genotypes are commonly found in local markets, the recommended rice genotypes can be immediately grown in local paddy fields to minimize As accumulation in rice grains, thus reducing the health risks of As exposure.

Screen for stable low-risk rice cultivars based on CDFOS and GGE

The ANOVA for the probability of grain As concentrations lower than the MPC in all tested environments is given in Table 2. The results indicate that the probability of grain As concentrations lower than the MPC was significantly affected by environment, genotype, and genotype–environment interactions. The contribution of variation was highest from the environment (80.75%), followed by genotype–environment interaction effects (11%) and genotype (1.67%).

Results of the GGE biplot analyses for the probability of grain As concentration lower than the MPC in all tested environments are given in Fig. 3. PC1 and PC2 explained 78.44% of the variability. According to the mean vs. stability view of the GGE biplot (Fig. 3A), the probability of grain As concentrations exceeding the MPC was lowest for TCS17, followed by TCS10, TT30, KH139, and TC192. Among these five genotypes, TCS17 and TT30 were regarded as stable, low-risk genotypes because of their relatively high stability across the different environments. For the “which-won-where” analysis, the results showed that all environments could be divided into six groups; however, four of these six groups had no tested environment (Fig. 3B). For the two environment groups consisting of at least one environment vector, TK14, TK16, and TCS17 were regarded as stable low-risk genotypes in each group. The discriminating power analysis showed that DC10IH, MS10IM, BT07I, DS09IH, and MS10IH were the most discriminating environments regarding the genotypes (Fig. 3C). Thus, these environments were ideal for genotype selection considering the probability of grain As concentration lower than the MPC among the tested environments.

Table 2 Analysis of variance for the probability of grain As concentration being lower than the threshold value (i.e., $0.35 \text{ mg As kg}^{-1}$ brown rice) at 18 environments of Taiwan

Sources of variation	DF	SS	MS	F value	P	%SS ^a
Environment (E)	17	107.3271	6.31336	327.62	<0.001	80.75
Genotype (G)	11	2.2140	0.20127	10.44	<0.001	1.67
G×E	187	14.6157	0.07816	4.06	<0.001	11
Residual	454	8.7488	0.01927			
Total	669	132.9055				

^aSum of squares (SS) of each effect by total SS

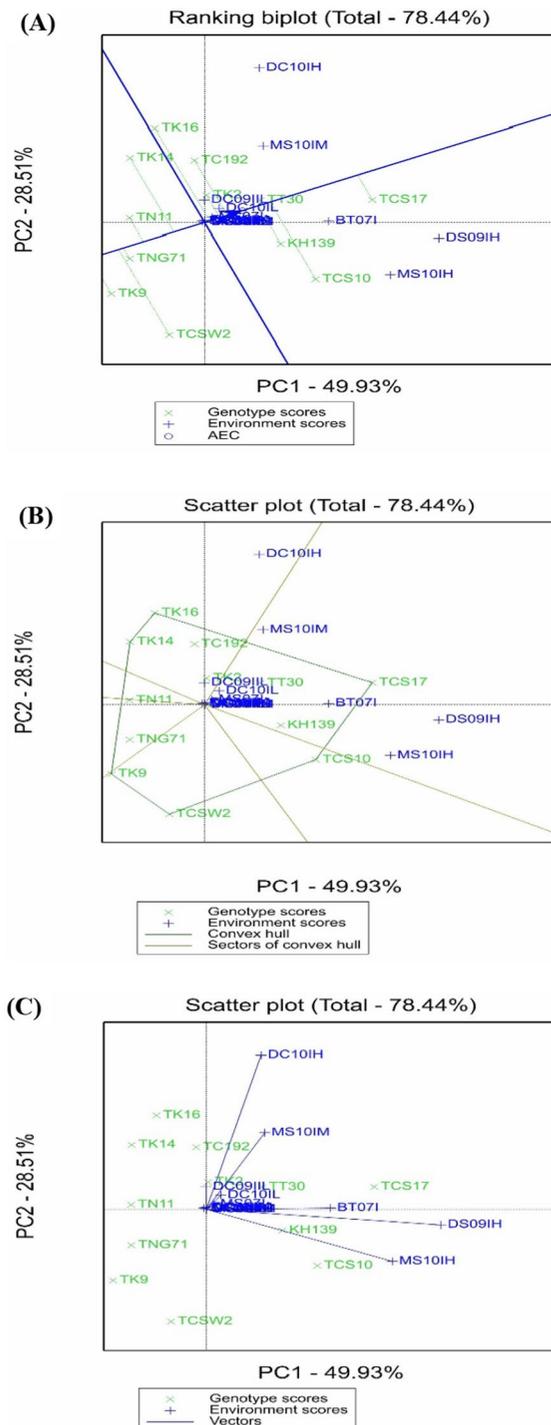


Fig. 3 The GGE biplots of the probability of As concentration in brown rice being lower than the MPC across all environmental trails to show **A** “Mean vs. Stability” view; **B** “Which-Won-Where” view; **C** “discriminating power” view. Green symbols are codes for rice genotypes. Blue symbols are codes for tested environments

The dataset of As concentrations in grains grown in multiple contaminated environments might be highly variable and skewed. For the protection of consumers’ health, the extreme values of As concentration in rice grains are usually of greater interest than the mean or median values. Therefore, some geostatistical approaches, such as disjunctive kriging or indicator kriging, are used to account for the probability of exceeding a critical threshold (Brus et al., 2002). Owing to its fewer requirements regarding the distribution of the raw data, rank-order geostatistics is an alternative for dealing with highly skewed data (Juang et al., 2001). This approach has been successfully applied for the determination of the probability of pollutant concentrations being lower than the threshold (Juang et al., 2001, 2005). In the present study, the winning genotypes selected by the combination of CDFOS and the GGE biplot were the same as those by the GGE biplot alone; however, the ranking of the genotypes was a little different. For example, the genotype KH139 ranked second when applying the GGE biplot, whereas it ranked fourth for the combination of CDFOS and the GGE biplot. This inconsistency may be due to the high skewness of grain As concentrations in KH139 across the tested environments. Thus, compared to the GGE biplot approach alone, the combination of CDFOS and the GGE biplot is a better choice for the screening of stable, low-As-risk genotypes, especially when the dataset is highly skewed.

Arsenic intake from rice has become a global concern, hence the maximum permissible concentration (MPC) of As in rice grains might be different in different countries (Mandal et al., 2021, 2023). The MPC of total As in rice grains proposed by the Joint FAO/WHO Expert Committee on Food Additives, Uruguay, and Brazil is 0.3 mg kg^{-1} (dos Santos et al., 2021; Roel et al., 2022; Toledo et al., 2022). However, the MPC of grain As in most countries is based on iAs rather than total As because the former is more toxic and has been classified as a Class A human carcinogen (Islam et al., 2016; Juang et al., 2022). For example, the Joint FAO-WHO Codex Alimentarius Commission has recommended a MPC of 0.2 mg kg^{-1} for iAs in polished rice and 0.35 mg kg^{-1} in husked rice (Mandal et al., 2021, 2023). In China, European Union, and Taiwan, there is no MPC for total As in rice grains, whereas the MPC for iAs in brown rice is 0.20, 0.25, and

0.35 mg kg⁻¹ in China, European Union, and Taiwan, respectively (Islam et al., 2016; Juang et al., 2021; Mu et al., 2019). In a previous study, the iAs level was reported to be in proportion to the total As in rice grains for the same 12 genotypes used in this study (Juang et al., 2022). Chi et al. (2018) also found strong correlations between concentrations of iAs and total As in the grains of 51 rice genotypes grown on four paddy fields in China. These results imply that it is reasonable to directly project the MPC of iAs to that of total As. Taking P_{inorg} (0.75) and the current local regulation for iAs in brown rice (0.35 mg kg⁻¹) into consideration, the MPC for total As is set to be 0.47 mg kg⁻¹ in this study. In fact, the inherent characteristic of order statistics is to take into account the rank of data rather than the magnitude of their differences. Therefore, the probability of grain As levels being lower than the threshold was reliably estimated using the combination of the rank-order statistical and GGE biplot approaches.

Human health risk assessment associated with rice consumption

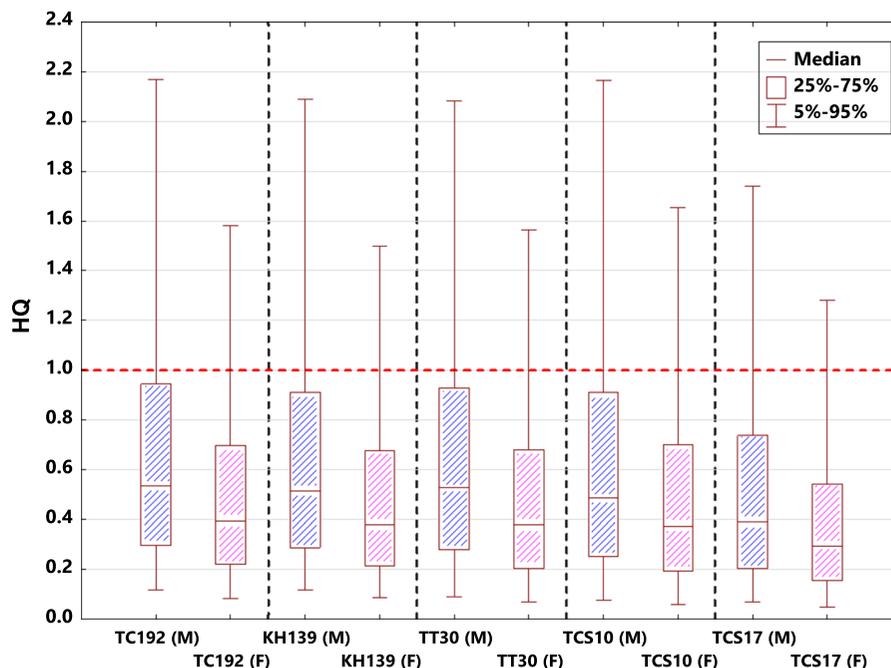
A human health risk assessment was conducted for the five genotypes selected by the combination of the GGE biplot and CDFOS analysis, i.e., TCS17, TCS10, TT30, KH139, and TC192. To determine the risk of local residents' rice consumption, the total As concentrations in brown rice of the selected genotype

in all tested environments were pooled together and then fitted by lognormal distribution to avoid unreasonable negative values during the simulation. The other parameters and input values in Eqs. (4) and (5) were obtained from the literature or official reports by the local government. Considering the high gender variation of *IR* and *BW*, the health risks of female and male populations were estimated separately. All parameters and input values used for the risk calculation are summarized in Table 3, whereas the simulation results of HQ are presented in Fig. 4. Generally, the HQ for male groups was higher than for female groups. This is mainly because the ingestion rate of male groups is greater than that of female groups. The median HQ value ranged from 0.293 (TCS17, female) to 0.534 (TC192, male), suggested that, on average, the health risk associated with consuming the selected rice genotypes was acceptable for local residents. Furthermore, the upper 75th percentile values of HQ were all less than unity, implying the suitability of these genotypes to be considered as stable, low-As-risk genotypes across all tested environments. When considering the worst situation (i.e., the upper 95th percentile value from the mean), however, potentially adverse impacts on human health would occur because all HQ values exceeded unity. In addition, the proportion of HQ values exceeding unity was highest for male consumers of genotype TCS10 (21.38%) and lowest for female consumers of genotype TCS17 (8.1%).

Table 3 Parameters and input values used in assessing human health risk of iAs associated with rice consumption

Parameter	Symbol	Input value	Unit	Source
Total As concentration in brown rice	C_{rice}		mg kg ⁻¹	This study
TC192		LN(0.58, 1.21)		
KH139		LN(0.46, 1.25)		
TT30		LN(0.48, 1.29)		
TCS10		LN(0.35, 1.47)		
TCS17		LN(0.31, 1.39)		
Ingestion rate	<i>IR</i>		g day ⁻¹	FDA (2022)
male		LN(109.18, 1.82)		
female		LN(63.87, 1.87)		
Proportion of iAs of total As in brown rice	P_{inorg}	0.75	-	Mandal et al., (2021, 2023)
Proportion of iAs of white rice to that in brown rice	$P_{white/brown}$	0.65	-	Juang et al. (2022)
Body weight	<i>BW</i>		kg	MHW (2022)
male		N(75.4, 21.6)		
female		N(58.7, 14.7)		
Reference dose	RfD	0.0003	mg kg ⁻¹ d ⁻¹	IRIS database

Fig. 4 Box–whisker plots of the estimated hazard quotient (HQ) of iAs for Taiwan residents associated with consuming white rice of selected genotypes. The blue box represents male population, whereas the pink box represents female population



As accumulation in rice grains has been recognized as a major concern for human health (Islam et al., 2016). Therefore, permissible As levels in rice have been continuously revised for human health issues in recent years (Roel et al., 2022). In the present study, the rice genotypes selected based on food safety standards were further examined by a human health risk assessment. Generally, the median HQ values were low; however, small proportions of HQ values were higher than unity. This result reflects that variability, especially toward the higher end, is larger. TCS17 was found to be the safest rice genotype in terms of the potential health risk of dietary As exposure. This is consistent with the results obtained by the GGE biplot and the combination of CDFOS and the GGE biplot analysis. The proportion of HQ exceeding unity for female and male consumers of genotype TCS17 was 8.1% and 13.79%, respectively, indicating that about 90% of consumers may experience negligible risks from dietary As intake through rice consumption of the selected rice genotype. It should be noted that the evaluated HQ values were obtained under the scenario of soil As contamination. In natural conditions, Fang et al. (2014) reported that 2.17% of polished rice samples exceeded the As limit based on the investigation of rice collected from different regions of China. Thus, it is unreasonable to

expect zero risk even for rice cultivated in soils with background As levels. However, Chen et al. (2016) calculated the risk of daily intake of iAs from rice collected from local markets in Taiwan and proposed that mean HQ values for female and male consumers were 0.11 and 0.15, respectively, which were lower than in the present study (female: 0.267, male: 0.356). Again, this may be due to the difference in soil As levels for rice cultivation. Another possible reason may be attributed to the overestimation of the risk due to the high uncertainties associated with them. Generally, the values obtained from the deterministic approach are lower than those from the probabilistic approach because the uncertainties and variability of data are not taken into consideration in the deterministic approach.

The sensitivity analysis showed that C_{rice} and IR were the major factors affecting health risks, while the contribution of BW was small, as shown in Fig. 5. Therefore, the effect of the higher C_{rice} by males in the present study overwhelmed that of the greater BW, so a larger HQ was found for males. This is consistent with many previous studies that have shown that the male population is much more susceptible to health risks from iAs ingestion compared to females (Chen et al., 2016; Juang et al., 2022). The higher sensitivity of C_{rice} suggests that it will be

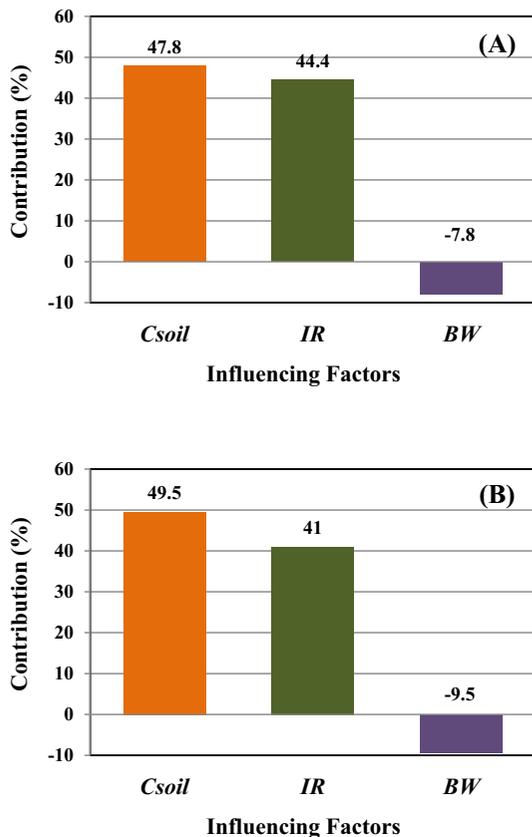


Fig. 5 Sensitivity analysis of hazard quotient (HQ) for local **A** female population and **B** male population associated with consuming white rice of selected genotypes

most efficient to minimize the risk of local residents exposed to iAs via rice consumption by reducing the As concentration in rice grains. Generally, there are two major ways to achieve this: better management practices to limit As entering rice and the screening and breeding of low-As-accumulating rice genotypes (Chi et al., 2018; Norton et al., 2009). For example, the supplementation of silicate materials and gene modification has been recommended as promising and effective techniques to reduce As accumulation in rice grains (Juang et al., 2021). The ingestion rate of white rice (IR) is another important factor in oral iAs exposure and varies widely from country to country. Theoretically, the risk of local residents exposed to iAs can be reduced by reducing the ingestion rate of white rice. In reality, a reduction in rice consumption is difficult because rice is the staple cereal consumed by local residents. Recently, it was reported that the

treatment of rice grain rinsing followed by boiling in excess water can reduce the bioaccessibility of As in rice grains, thus reducing health risk of iAs associated with rice consumption (Sharafi et al., 2019). In the present study, a comprehensive methodology was employed to screen for stable, low-As rice genotypes across multiple field environments in Taiwan. Due to their superior performance based on food safety and health risk perspectives, the selected rice genotypes could be immediately cultivated in local paddy fields for the protection of consumers' health.

Research limitations and implications

In the present study, the combination of CDFOS and the GGE biplot analysis was developed and used to screen for stable, low-As-accumulating rice genotypes. It should be noted, however, that the results obtained here were based on the experimental data of the selected rice cultivars and environmental conditions. Several site-specific geochemical factors such as pH, water contents, iron plaque formation, and mineral elements levels of the studied soils could affect As uptake by rice (Ahmed et al., 2011; Chi et al., 2018; Juang et al., 2021). This implies that the selection of studied sites and environmental conditions is crucial while developing current methodology. When more trial environments were included in the analysis, it might result in rank changes of cultivars for grain As levels across the extensive trial environments. The rice cultivars selected here might not always show significant lower As accumulation at different sites and different years. In view of this, more extensive and in-depth researches are encouraged in the future to mitigate the variabilities and enhance the stability of cultivars selection.

When assessing the risk of As entering the food chain through rice consumption, the ratio of iAs to total As in brown rice (P_{inorg}) is a primary parameter. It was reported that large environmental variation exists of this parameter (Norton et al., 2009). According to Mandal (2021; 2023), the P_{inorg} ranged from 0.53 to 0.97 in Asian countries. In the present study, the P_{inorg} was set to be 0.75 according to the mean value proposed by Mandal (2021; 2023). In our previous study, the P_{inorg} was calculated to be 0.68 for the same 12 genotypes used in this study (Juang et al., 2022). In this regard, the human health risk might be

overestimated in the present study. A detailed study on how P_{inorg} varies within genotypes across multiple environment is thus necessary to reduce the uncertainty in the health risk assessment. On the other hand, As intake via rice consumption was only one exposure route. Local residents might be exposed to As through drinking water and seafood. Moreover, it should be noted that the reference dose (RfD) of iAs used in the present study is for normal adult population. A decrease in RfD value maybe expected for sensitive subpopulations or children, thus resulted in an increase of health risk. To overcome these limitations, therefore, further research work should be dedicated to the diet structure of local residents and the establishment of dose–response relationship of iAs for sensitive subpopulations.

In order to examine the food quality standard for As, different values of C_{rice} , with all parameters and input values in Table 3, were introduced into Eq. (4). Based on the simulation results, the median values of HQ were all less than unity for both genders when the value of C_{rice} was lower than 0.4 mg kg^{-1} . On the other hand, the upper 75th percentile values of HQ were all less than unity when the value of C_{rice} was lower than 0.26 mg kg^{-1} . From the viewpoint of human health risk, therefore, the total As in rice grains is advised to fall below 0.4 mg kg^{-1} . However, other aspects including social and economic consideration should be also included by policymakers while setting food quality standard for As in rice grains.

Conclusions

In this study, multiple-environment trials of As accumulation in the grains of 12 rice genotypes were conducted at four As-contaminated paddy fields in Taiwan. First, the experimental data were analyzed by GGE biplot to identify stable, low-As-accumulating genotypes. Second, rank-order statistics were incorporated into the GGE biplot to evaluate the probability of As concentrations exceeding the MPC for the selected genotypes. Five rice genotypes were successfully selected for the alleviation of As contamination in rice grains. Third, a human health risk assessment was employed to characterize the risk posed by rice consumption of the selected rice genotypes. Among all tested genotypes, TCS17 was recommended as the ideal rice genotype for the mitigation of risk

associated with rice consumption by local residents. In conclusion, a comprehensive methodology was developed in this study to make a practical recommendation for stable, low-As-risk rice genotypes by taking into account the environment–genotype interaction, food quality standards, and a health risk assessment.

Acknowledgements This research was financially supported by the National Science and Technology Council, Taiwan, Republic of China, under grant Nos. MOST 108-2313-B-343-001-MY3 and MOST 111-2313-B343-001-.

Author contributions All authors contributed to the study conception and design. Conceptualization and methodology were performed by [B-CC] and [K-WJ]. Experiment, investigation, resources, and data analysis and curation were performed by [K-WJ], [TT], and [C-HS]. The first draft of the manuscript was written by [B-CC], and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Funding This work was financially supported by [National Science and Technology Council, Taiwan, Republic of China] (Grant numbers [MOST 108–2313-B-343–001-MY3] and [MOST 111–2313-B343-001-]).

Data availability The data used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of interest The authors have no relevant financial and non-financial interests to disclose.

Ethical approval The authors confirm that the Committee on Publication Ethics (COPE) guidelines have been adhered to in the submission of this manuscript.

Consent to participate Not applicable.

Consent to publish The authors all agree to the publication of this journal. No other consents for publication were required.

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