



Mineral nutrients and heavy metal evaluation and their relationship with morpho-agronomic traits in new rice varieties with *qDTYs* and *Sub1* QTL

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Malaysia has released over 55 rice varieties, but none are high in mineral nutrients, especially zinc and iron. Furthermore, some rice varieties with high nutrient content and high yields can be contaminated with heavy metals such as arsenic and cadmium. This study examined the concentration of mineral nutrients and heavy metals in polished and unpolished rice with morpho-agronomic traits and with *qDTYs* and *Sub1* in the genotypes. Five genotypes viz. UKM5, UKM112, UKM37, UKM54, and UKMRC9 were field-tested using a completely randomised design with four replications. After one planting season, morpho-agronomic data was collected. The harvested grain was processed and analysed for mineral nutrients and heavy metal content. In this study, unpolished rice has more Zn, Fe, and As, while polished rice has more Cd. UKM112 rice has the best morpho-agronomic traits, high mineral nutrient content, and low metal content, especially after being polished. The combination of *qDTY_{3.1}* and *Sub1* increased morpho-agronomic traits, mineral nutrients, and heavy metals, while *qDTY_{12.1}* and *qDTY_{3.1}* had the opposite effect. This study also found that while mineral nutrients boost grain yield and plant growth, heavy metals may also accumulate under specific conditions. This study can have a significant impact on the problems of malnutrition and rice income faced in Malaysia. The selected rice genotype can also be used as a donor parent to produce more rice varieties that are high in mineral nutrients, low in heavy metals, and high-yielding for Malaysia.

Keywords: Biofortification, Cadmium, Arsenic, Zinc, Ferum, Nutrition.

Introduction

Rice is a staple food for half the world's population, especially in the largest rice-producer countries such as Indonesia, India and China. Rice is one of the primary sources of carbohydrates and protein globally, including in Malaysia (Dorairaj and Govender, 2023). However, some varieties of rice have low sources of mineral nutrients such as zinc (Zn) and ferum (Fe) in seeds, especially when polished. Several varieties of coloured rice have been produced but are often sold as special rice at high prices due to its high nutrient and vitamin content (Dwivedi *et al.*, 2023; Napasintuwong, 2020).

Fe and Zn are essential minerals for normal and healthy growth especially in children, pregnant mothers, and breastfeeding mothers (Hefferon, 2019). Malnutrition of mineral nutrients Fe and Zn

has affected over 3 billion people worldwide, mostly in developing countries. It is because of the extreme poverty that strikes many people. Almost 1.5 billion people live in extreme poverty, 80% of whom live in developing countries. Not only that, the poor also have little or no access to proper health and education. This means that those living in extreme poverty are five times more likely to die before the age of five, and two and a half times as likely to die between the ages of 15 and 59, compared to those in the high-income group (Peña and Bacallao, 2002).

In addition to Fe and Zn, deficiencies of other mineral nutrients such as selenium (Se), calcium (Ca), magnesium (Mg) and copper (Cu) are also becoming more common in some parts of the world and have caused many health problems, especially

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in children. Malnutrition has been identified as a global health concern and one of the most significant challenges to the world (Dukhi, 2020; World Health Organization 2019; 2021), and should be given priority to meeting the Sustainable Development Goals to reduce child and pregnant mother mortality, as well as poverty and hunger.

Biofortification can be used to tackle malnutrition by increasing the mineral nutrients in staple foods (Wan Muda *et al.*, 2019). Through biofortification, many nutrient-rich varieties were released for cultivation in the field such as DRR Dhan 45 (Sanjeeva Rao *et al.* 2020), BRRI Dhan 102 (Bin Rahman and Zhang, 2023 and CR Dhan 31 (Chattopadhyay *et al.*, 2019). However, studies on the assessment of mineral nutrient content in rice varieties in Malaysia are very few and limited. No varieties of white rice in Malaysia have previously been declared to have a high mineral nutrient content, especially Zn and Fe. The selection and production of suitable varieties are crucial in dealing with malnutrition among communities.

Behind the production of high mineral nutrient varieties, several previous studies have found a negative correlation between grain yield and mineral nutrients, especially Zn (Calayugan *et al.*, 2020; Descalsota-Empleo *et al.*, 2019a; Descalsota-Empleo *et al.*, 2019b; Swamy *et al.*, 2018a; Swamy *et al.*, 2018b. In addition, as a significant source of nutrients, rice can also be a source of toxic heavy metals such as arsenic (As) and cadmium (Cd) due to the excessive use of chemical fertilizers and pesticides by farmers. According to studies conducted in China, rice can contribute up to 60% of arsenic intake and 56% of cadmium intake in the daily diet of populations consuming contaminated rice (Li *et al.*, 2011; Song *et al.*, 2017). Exposure to these heavy metals can cause a variety of health effects on humans. Therefore, it is vital to ensure that the content of heavy metals can be minimized alongside increasing the concentration of mineral nutrients to produce nutritious and safe-to-eat rice. This study was conducted with the aim of evaluating the relationship between mineral nutrients and heavy metal in polished and unpolished rice with the morpho-agronomic traits, evaluating the relationship between the presence of *qDTYs* and *Sub1* in rice genotypes with the morpho-agronomic traits and accumulation of mineral nutrients and heavy metals in grain and finally to select genotypes with high accumulation of mineral nutrients and low accumulation of heavy metals.

Materials and Methods:

Plant materials

Five rice varieties, viz., UKM5, UKM54, UKM112, UKM37, and UKMRC9 were used in this study.

UKMRC9 produces red-coloured pericarp grain and was used as the control variety as it was found to have a high zinc content. The other four varieties produce white-coloured pericarp grain. All varieties were developed through marker-assisted breeding techniques. The information on all varieties is listed in Table 1. The seeds of the varieties were obtained from UKM Rice Genebank facilities.

Table 1. Varieties used in the study and their respective information.

| Varieties | Development strategy | QTL | Characteristics |
|-----------|--|---|----------------------------------|
| UKM5 | QTL pyramiding into MR219 | <i>qDTY_{12.1}+qDT_{Y3.1}</i> | Drought tolerant |
| UKM37 | Backcrossing of UKM91 and IR64-Sub1 | <i>Sub1</i> | Submergence tolerant |
| UKM54 | Backcrossing of UKM91 and IR64-Sub1 | <i>Sub1</i> | Submergence tolerant |
| UKM112 | Backcrossing of UKM5 and IR64-Sub1 | <i>Sub1+qDTY_{3.1}</i> | Drought and submergence tolerant |
| UKMRC9 | Backcrossing of MR219 and <i>Oryza rufipogon</i> | - | Red-coloured pericarp, high zinc |

Experimental design and cultural practices

The study was conducted in Kuala Terengganu, Malaysia, between October 2022 and March 2023. The experiment was conducted in a randomized complete block design with four replications. For each replication, the plot size was 1 m × 2 m with a planting distance of 25 cm between rows and hills. Fourteen-day-old seedlings were transplanted into the field at the rate of one seedling per hill, resulting in 45 individual plants in each replication. Standing water of 5 cm was maintained throughout the growing period. The application of pesticides and fungicides to pests and diseases was carried out based on the recommendations of the Department of Agriculture Malaysia.

Phenotyping

The Standard Evaluation System of Rice (IRRI, 2013) procedure was used for collecting data. Days

to 50% flowering (DTF) were recorded from the transplanting date until 50% of the plants had flowered. For each genotype in each replication, 40 plants were measured for plant height, recorded from the base of the plants to the tip of the longest panicle. For each plant, the number of panicles (NP) was counted, and 10 representative panicles were taken for measurement of the length of the panicle (PL). The number of grains per panicle (GPP) was counted by hand, and 100 grains were weighed to record the hundred-grain weight (HGW). All grains were separated from the panicles, dried in the oven for two days, and weighed to obtain the grain yield (GY). The weights were adjusted to 14% moisture content for analysis.

Nutrient and Heavy metal analysis

The harvested grains from each genotype were dried in the oven at 65 °C for 48 hours to a constant weight. Half of the sample for each replicate and each genotype was polished using the lab-scale grain polisher for 1 min to obtain the polished rice grain while the other half was not polished. Approximately 1.0 g sample was added into 10 mL nitric acid and digested using the microwave digester (Milestone Start D Microwave Digestion System). The digestion follows the procedure outlined in Kumar *et al.* (2022). After digestion, the sample was diluted with mili-Q water in the Eppendorf tube to 50 mL total volume and analysed using the inductively coupled plasma mass spectrometry (ICP-MS) [NexION 2000, Perkin Elmer]. The standard solution from Agilent was used as the reference. The concentration of grain mineral nutrients (Ferum, Zinc) and heavy metals (Arsenic, Cadmium) was measured in parts per billion and converted to ppm (equal to mg/kg) using the following formula:

$$\text{Concentration} \left(\frac{\text{mg}}{\text{kg}} \right) = \frac{\text{Concentration in ppb}}{1000} \times \frac{50 \text{ (mL)}}{1.0 \text{ g}}$$

Statistical Analysis

Descriptive statistics and one-way analysis of variance (ANOVA) were performed using the *aov* function in the *stats* package (R Core Team, 2020). Pearson's correlation coefficient values were calculated using the *stats* package and visualized using the *corrplot* package (Wei and Simko, 2017). The principal component analysis (PCA) was carried out using the *stats* and *ggbiplot* packages (Vu, 2011). All analyses were carried out in RStudio version 1.2.5003 (RStudio Team, 2020).

Results

Descriptive statistics and coefficient of variation analyses

The highest coefficient of variation (CV) was recorded for the concentration of cadmium in

polished grain (GCdp), followed by grain yield (GY) and number of panicles (NP). The lowest CV was recorded for days to 50% flowering (DTF), followed by hundred-grain weight (HGW). The maximum concentration of ferum in unpolished grain (GFeb), concentration of zinc in unpolished grain (GZnb) and concentration of zinc in polished grain (GZnp) were found in UKM5. In contrast, the maximum concentration of ferum in polished grain (GFep) was found in UKM37. Meanwhile, the lowest concentration of arsenic in polished grain (GAsp), concentration of arsenic in polished grain (GAsp), and concentration of cadmium in unpolished grain (GCdb) and GCdp were found in UKM5. The descriptive statistics results for all traits are presented in Table 2.

Analysis of variance (ANOVA)

The ANOVA showed significant differences due to the effect of genotypes in DTF, PH, NP, LP, and GPP. There was no significant effect of genotypes in the concentration of ferum in both polished and unpolished grain (GFeb and GFep) (Figure 1). The same result was observed in the concentration of zinc in polished grain (GZnp). However, a significant difference in the concentration of zinc in unpolished grain (GZnb) was observed among the genotypes tested (Figure 2). For both the concentration of cadmium and arsenic in polished grain (GCdp and GAsp), the effect of genotypes is significant (Figures 3 & 4). A significant difference between genotypes was observed in the concentration of arsenic in unpolished grain (GAsb) but not in the concentration of cadmium in unpolished grain (GCdb) (Figures 3 & 4).

Based on Table 3, all genotypes have one to two days of difference in DTF except for UKM5 which possesses the combination of *qDTY_{12.1}* and *qDTY_{3.1}* recorded the earliest DTF, which was a six-day difference compared to UKM54. The PH of all genotypes was below 100 cm. UKM112 which possesses the combination of *Sub1* and *qDTY_{3.1}* recorded the highest number of panicles followed by UKM54. The earliest flowering genotype, UKM5 however recorded the lowest number of panicles. Its panicles also were the shortest compared to the other genotypes. Nevertheless, the GPP of UKM5 was the highest of all the genotypes tested. UKM112 recorded the highest yield among all genotypes, even though it was not statistically significant.

The GZnb of UKM5 was the highest, followed by UKM37 and UKM54. The GAsb, GAsp and GCdp of UKM5 also were the lowest compared to the other genotypes. As shown also in Figure 4, genotypes such as UKM54 and UKM112 recorded higher concentrations of cadmium in polished rice compared to unpolished rice.

Pearson correlation analysis

Figure 5 shows the results of the correlation analysis of mineral nutrients, heavy metals and morpho-agronomic traits. The analysis results show that GY is positively correlated with GAsb, ($r = 0.54$, $p < 0.01$), GFep ($r = 0.43$, $p < 0.01$), GZnp ($r = 0.13$, $p > 0.05$), GAsp ($r = 0.41$, $p < 0.01$) and GCdp ($r = 0.42$, $p < 0.05$) but negatively correlated with GFeb ($r = -0.14$, $p < 0.05$), GZnb ($r = -0.22$, $p < 0.01$) and GCdb ($r = -0.13$, $p > 0.05$). In addition, PH was positively correlated with GFep ($r = 0.27$, $p < 0.05$), GAsp ($r = 0.5$, $p < 0.05$), GCdp ($r = 0.06$, $p < 0.05$), GAsb ($r = 0.29$, $p < 0.05$) and GCdb ($r = 0.24$, $p > 0.05$) but negatively correlated with GFeb ($r = -0.19$, $p > 0.05$), GZnp ($r = -0.22$, $p > 0.05$) and GZnb ($r = -0.54$, $p < 0.001$). Furthermore, LP is positively correlated with GAsb, GCdb, GFep, GAsp and GCdp but negatively correlated with GFeb, GZnb and GZnp. GZnp is positively correlated with GFep ($r = 0.33$, $p > 0.05$), while GZnb is also positively correlated with GFeb ($r = 0.45$, $p < 0.05$). LP negatively correlated with GFeb, GZnb and GZnp. Meanwhile, GY is positively correlated with PH, NP, LP and GPP but negatively correlated with HGW.

Days to 50% flowering (DTF), plant height (PH) in cm, number of panicles (NP), length of panicle (LP) in cm, number of grain per panicle (GPP), hundred-grain weight (GWG) in g, grain yield (GY) in kg ha⁻¹, concentration of ferum in unpolished grain (GFeb), concentration of zinc in unpolished grain (GZnb), concentration of arsenic in unpolished grain (GAsb), concentration of cadmium in unpolished grain (GCdb), concentration of ferum in polished grain (GFep), concentration of zinc in polished grain (GZnp), concentration of arsenic in polished grain (GAsp), concentration of cadmium in polished grain (GCdp)

Table 2. Descriptive statistics of agro-morphological traits, grain mineral nutrient and heavy metal concentration.

| Trait | Mean \pm SE | CV (%) | Minimum | Maximum |
|-------|----------------------|--------|---------|---------|
| DTF | 76.80 \pm 0.7310 | 4.26 | 70.00 | 80.00 |
| PH | 91.88 \pm 1.4500 | 7.08 | 79.80 | 102.60 |
| NP | 10.18 \pm 0.7880 | 34.61 | 4.90 | 21.78 |
| LP | 23.94 \pm 0.3710 | 6.93 | 21.28 | 26.78 |
| GPP | 133.75 \pm 2.8100 | 9.38 | 115.40 | 158.50 |
| HGW | 2.66 \pm 0.0346 | 5.82 | 2.29 | 2.93 |
| GY | 3656.00 \pm 312.00 | 38.16 | 1774.00 | 8347.00 |
| GFeb | 15.40 \pm 0.5040 | 14.64 | 12.83 | 22.61 |
| GZnb | 19.34 \pm 0.4350 | 10.06 | 16.34 | 23.05 |
| GAsb | 0.16 \pm 0.0090 | 24.89 | 0.12 | 0.24 |
| GCdb | 0.02 \pm 0.0005 | 14.74 | 0.01 | 0.02 |
| GFep | 8.99 \pm 0.5300 | 25.65 | 3.59 | 13.72 |
| GZnp | 14.81 \pm 0.3510 | 10.32 | 9.95 | 17.63 |
| GAsp | 0.12 \pm 0.0069 | 25.37 | 0.06 | 0.18 |
| GCdp | 0.02 \pm 0.0033 | 73.31 | 0.01 | 0.08 |

Table 3. Mean values of agro-morphological traits for each genotype.

| Genotype | QTL | DTF | PH | NP | LP | GPP | HGW | GY |
|----------|--|---------|-----------|----------|----------|-----------|--------|-----------|
| UKM112 | <i>Sub1+qDTY_{3.1}</i> | 80.00 a | 98.43 a | 14.00 a | 25.07 ab | 126.65 b | 2.53 a | 4616.99 a |
| UKM37 | <i>Sub1</i> | 78.00 b | 85.38 c | 9.00 ab | 23.49 b | 122.60 b | 2.73 a | 3104.90 a |
| UKM54 | <i>Sub1</i> | 77.00 b | 93.15 abc | 11.00 ab | 25.56 a | 129.59 b | 2.76 a | 3563.97 a |
| UKM5 | <i>qDTY_{12.1}+ qDTY_{3.1}</i> | 71.00 c | 86.88 bc | 7.00 b | 21.47 c | 152.00 a | 2.72 a | 2936.16 a |
| UKMRC9 | - | 78.00 b | 95.55 ab | 11.00 ab | 24.11 ab | 137.93 ab | 2.56 a | 4056.60 a |

Days to 50% flowering (DTF), plant height (PH) in cm, number of panicles (NP), length of panicle (LP) in cm, number of grain per panicle (GPP), hundred-grain weight (HGW) in g, grain yield (GY) in kg

ha⁻¹. Mean values with the same letter for each trait are not significantly different by Tukey's HSD test ($p > 0.05$).

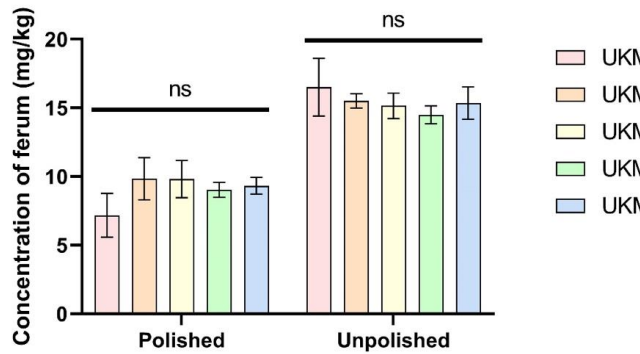


Fig. 1. Concentration of ferum in the grain of polished and unpolished rice.

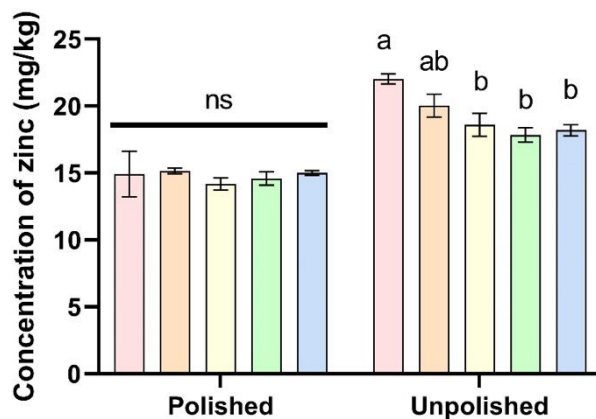


Fig. 2. Concentration of zinc in the grain of polished and unpolished rice.

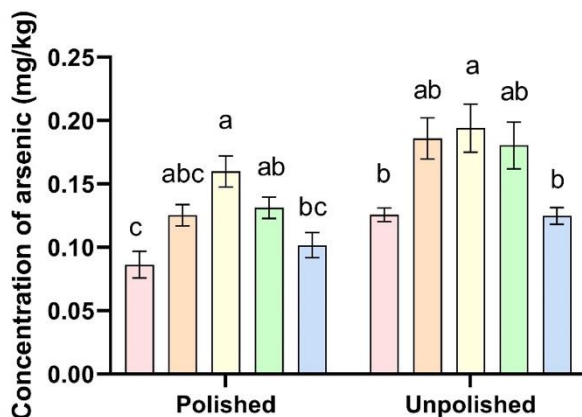


Fig. 3. Concentration of arsenic in the grain of polished and unpolished rice.

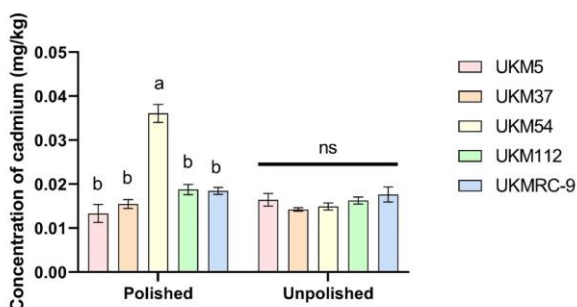


Fig. 4. Concentration of cadmium in the grain of polished and unpolished rice.

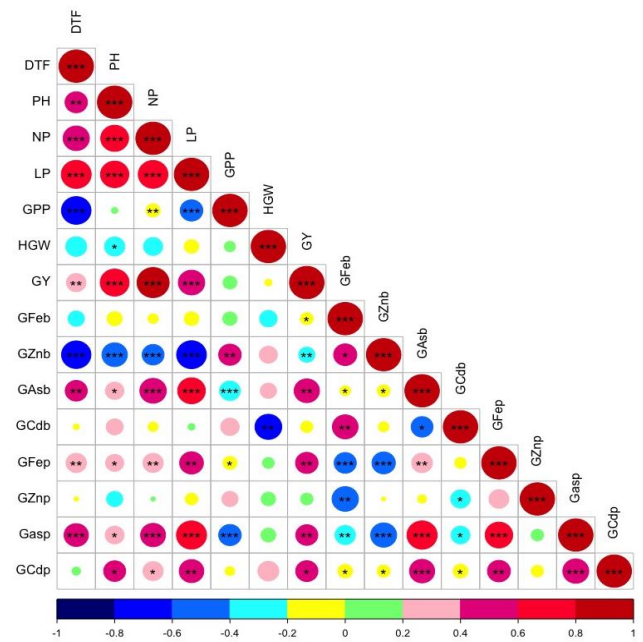


Fig. 5. Matrix of correlation among all traits recorded. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Discussion

The type of rice affects the variation in mineral nutrient content and heavy metal levels. The results of the t-test analysis showed that the mean per genotype for mineral nutrients and heavy metals namely Zn, Fe, and As in unpolished rice was higher than polished rice. The disparity in mineral nutrients and heavy metals between the two types of rice is due to the polishing process. Rice grains comprises of several layers, including husks, bran, germ, and endosperms. The outer layers (husk, bran, and germ) are rich in minerals, vitamins, and dietary fibre, while the endosperm contains starch (Juliano and Faano, 2019). The rice polishing process is the process by which the outer layer of rice grains is removed to produce white rice. This process involves grinding using a machine to remove the husks, bran, and germs, leaving only the starchy endosperm and producing white rice that has a smooth and shiny appearance (IRRI, 2023). The outer layers, i.e., husk, bran, and germ, in rice grains contain higher concentrations of mineral nutrients and metals, including Fe, Zn, Mg, and As. Therefore, when the outer layer is removed during the milling process, most of the mineral and heavy metal content is lost, leaving behind the endosperm part known as polished rice. The more polished the rice, the greater the loss of mineral nutrients and heavy metals (Varma *et al.*, 2019). Therefore, polished rice tends to have a higher content of Fe, Zn, and As than unpolished rice. Polished rice also accumulates more Cd, concentrated in the rice endosperm than in other layers (Tian *et al.*, 2014). According to Tian *et al.* (2014), Cd is bound to

protein, which is stored in the endosperm. In another study, Cd is found to be more accumulated in the centre of grain through autoradiography (Hirose *et al.*, 2014).

The results also showed that the content of minerals nutrients and heavy metals affected rice grain yield and other morpho-agronomic traits. GY, PH and LP have a positive correlation for both mineral nutrients. This shows that mineral nutrients help in increasing rice grain yield and rice growth. Fe plays a crucial role in metabolic processes such as photosynthesis, chloroplast development, chlorophyll biosynthesis, electron transport, and redox reactions. All these physiological processes are important in increasing grain yield and stimulating rice growth (Aung and Masuda, 2020). Zn also stimulates physiological and metabolic processes, enhancing grain yield (Sudhagar *et al.*, 2019).

Meanwhile, Cd and As also positively correlated with GY, PH and LP. According to Kanu *et al.* (2017), increased Cd levels in plants can inhibit the use, uptake and transport of nutrients and water and modify the mechanism of photosynthesis. This can result in the death of plant tissues and reduce rice yield and grain quality. Arsenic can disrupt metabolic processes, inhibit growth, inhibit the growth of roots and shoots, and reduce rice grain production (Rahman, 2007). Notably, different studies have yielded varying results regarding the impact of Cd and As on grain yields, suggesting genotype-specific adaptations. This suggests that the genotype employed in the study exhibits favourable adaptability to the effects of Cd and As, resulting in increased grain yields and other morpho-agronomic traits.

The analysis of mineral nutrients, heavy metals, and morpho-agronomic traits showed significant differences between different genotypes. These variations are attributed to different QTLs (quantitative trait loci) in each genotype, influencing nutrient content and overall traits. The combined effects of *Sub1* and *qDTY_{3.1}* on the UKM112 genotype significantly influenced the mineral nutrient content, heavy metals, and morpho-agronomic traits of rice. This assertion is supported by the fact that the mean values of all traits in UKM112 were the highest compared to other genotypes. *Sub1*, a gene responsible for flood resistance, enables rice plants to survive and recover even when fully submerged. In contrast, *qDTY_{3.1}* is associated with drought tolerance during the reproductive stage of rice crops (Mohd Ikmal *et al.*, 2021). The synergistic action of these two quantitative trait loci (QTLs) positively impacts rice growth and the accumulation of mineral nutrients and heavy metals.

The combined effects of *qDTY_{12.1}* and *qDTY_{3.1}* on the UKM5 genotype diverged from those observed in UKM112. Mineral nutrient content, heavy

metals, and morpho-agronomic traits recorded for UKM5 are the lowest values compared to other genotypes. While the presence of *qDTY_{3.1}* with *Sub1* positively impacted nutrient content and morpho-agronomic traits, the co-occurrence of *qDTY_{12.1}* with *qDTY_{3.1}* yielded a contrasting effect. According to Dixit *et al.* (2015), *qDTY_{12.1}* enhances root development, aiding water and mineral nutrient absorption, particularly under drought conditions. Interestingly, when *qDTY_{12.1}* appears alone without other *qDTY* combinations, it influences root enhancement (Mohd Ikmal *et al.*, 2019). This suggests that the combination of these two quantitative trait loci (QTLs) acts as a barrier to the accumulation of mineral nutrients, heavy metals, growth, and rice yield.

Additionally, the UKM37 and UKM54 genotypes also exhibited high mineral nutrient content, heavy metals, and morpho-agronomic traits following UKM112. The presence of *Sub1* in these genotypes indicates its role in facilitating the accumulation of mineral nutrients, heavy metals, and morpho-agronomic traits. Bailey-Serres *et al.* (2010) demonstrated that varieties carrying the *Sub1* gene exhibit superior agronomic performance, including yield and grain quality under normal growth conditions. *Sub1* suppresses gibberellic acid (GA) synthesis and GA-based signalling pathways (Emerick and Ronald 2019), reducing shoot elongation. Consequently, rice plants allocate carbohydrates more efficiently toward grain yield expansion.

The selection of rice with the best genotype must consider the minerals nutrient content and heavy metals in rice grains. While it is impossible to eliminate heavy metals such as As and Cd from each grain, their levels can be mitigated through genotype selection and appropriate milling processes. Ensuring low heavy metal content in rice grains prevents health issues. Kong *et al.* (2018) recommend the following acceptable ranges for heavy metals in rice grains: As (0.03–0.192 mg/kg) and Cd (0.001–0.099 mg/kg) [1 mg/kg = 1 ppm]. Additionally, the Ministry of Health Malaysia (2023) specifies a maximum allowable Cd level of 0.4 mg/kg in rice.

Meanwhile, mineral nutrient content must meet recommended nutritional standards to prevent malnutrition. Bouis *et al.* (2011) propose optimal levels for Zn (24–28 mg/kg) and Fe (13 mg/kg). Based on the analysis of mineral nutrients and heavy metals in rice grains in each genotype, none exceeded hazardous thresholds and were sufficient for As, Cd and Fe content. However, all genotypes fell short of the recommended rate for Zn. This is due to the low availability of Zn in the soil compared to other mineral nutrients. Soil analysis, the Zn content in the soil is not per the standard issued by the Department of Environment (2009) which is 6.9 – 54.3 mg/kg.

Conclusions

Different QTL combinations impact mineral nutrients, heavy metal content, and morpho-agronomic traits in rice genotypes. The combination of QTL Sub1 and qDTY3.1 in UKM112 positively influences growth and nutrient accumulation, resulting in high values for these traits. Conversely, qDTY12.1 and qDTY3.1 in UKM5 have a negative impact, showing the lowest values. UKM112 is identified as the best genotype due to its favorable characteristics, which are good morphology, high mineral nutrient content, and low heavy metal content after polishing.

Consent for publication:

All authors declare their consent for publication.

Author contribution:

The manuscript was edited and revised by all authors.

Conflicts of Interest:

The author declares no conflict of interest.

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