Asian Research Journal of Agriculture

10(1): 1-14, 2018; Article no.ARJA.45221
ISSN: 2456-561X

Assessment of Some Heavy and Essential Elements Accumulation in Seeds and Leaves of Parent and Introgression Lines in *Oryza sp.*

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/ARJA/2018/45221
Editor(s):
(1) Dr. Rusu Teodor, Department of Technical and Soil Sciences, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Romania.
Reviewers:
(1) Jorge Isaac Castro Bedriñana, Universidad Nacional del Centro del Perú, Peru.
(2) Branislav Kovacevic, University of Novi Sad, Serbia.
Complete Peer review History: http://www.sciencedomain.org/review-history/27541

Original Research Article

Received 18 September 2018
Accepted 24 November 2018
Published 01 December 2018

ABSTRACT

In this study, element concentrations in grains of three wild rice species (i.e., *Oryza rufipogon, Oryza officinalis* and *Oryza meyeriana*) and three cultivated rice varieties (i.e., Nipponbare, 93-11 and HY-8) as well as in leaves of *Oryza officinalis* and its introgression lines were measured. The wild rice species were highly useful in terms of the quality and the harmful heavy metal resistance. The results showed that the mean concentrations of heavy metals in the soil was in the ranking order of Ti > Zr > Sr > Pb > Rb > As. The essential element concentrations, it was found that the

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ranking order was Ca > Fe > K > Mn > Zn > Cu > Mo. In grains, the content of Ca, Zn and Sr elements in wild rice species was higher than that of cultivated rice varieties. *Oryza meyeriana* had the highest content of Ca and Sr, and *Oryza officinalis* had the highest content of Zn. The levels of Mo were high in *Oryza rufipogon*, while extremely low in *Oryza officinalis* and *Oryza meyeriana*. *Oryza officinalis* had much higher Cu than other varieties. Bioaccumulation values of all elements were less than one in the rice grain. The concentrations of elements varied in different parts of rice plants, and the highest concentrations occurred in the leaves comparing with grains. The introgression lines i.e., FC7-12, FC7-6, FC7-20 and FC7-1 showed good performance and ability in heavy metals resistance. High genotypic and phenotypic variances were observed in Mn concentrations followed by Fe concentrations, respectively. This study preliminarily proves that there is genetic diversity in element absorption and accumulation among different genotypes of wild rice species, cultivated varieties and introgression lines. Wild rice species are useful for developing high quality and improving tolerance to heavy metals in modern rice cultivars.

Keywords: Rice; wild species; bioaccumulation factor; genetic variability; heavy metal tolerance.

1. INTRODUCTION

Rice has been grown as a major food crop and accounts for more than half of worldwide human food consumption [1]. With an ever-growing global population, especially in regions where rice is the dominant food source, the elemental quality of the grains is potentially a critical factor affecting upon human health. A large proportion of dietary minerals are obtained from grains. Improvement of mineral content in grains could, therefore, have a positive effect on human health. In this regard, increasing grain vitamin A, iron (Fe) and zinc (Zn) are considered at the highest priority [2]. Iron and Zn deficiencies are two of the major micronutrient deficiencies in the world, affecting an estimated 2 billion people worldwide [3,4]. It has been proposed that even a small increase in the Fe nutritional value of rice grain, in regions where rice is the staple, would be highly significant [5].

In the developing countries of Asia, such as the Philippines and China, cereals provide approximately 50% of iron and zinc intake [6,7]. The number of people affected by zinc deficiency in these two countries is estimated to be 86 million and 10 million, respectively, with most living in rural areas [7] including southwestern China. The micronutrients such as Zn, Mn, and Cu are required in small but critical concentrations for both plants and animals, and these have a vital role in physical growth and development of crop plants such as paddy.

Heavy metals in the environment are a health hazard due to their persistence, bioaccumulation and toxicity in plants, animals and human beings [8]. High concentrations of heavy metals in the soil would increase the potential uptake of these metals by plants. There is a tendency in plants to take up heavy metals that may subsequently transfer into the food chain. Use of polluted soil or water for crop cultivation mainly results in the decrease of overall productivity and contaminates food grains and vegetables, which adversely affect human health too [9].

It has also been reported that crop plants have different abilities to absorb and accumulate heavy metals in their different organs. There is a broad difference in metal uptake and translocation between plant species and even between cultivars of the same plant species [10]. Plants absorb heavy metals from the soil, and the surface 25 cm zone of soil is mostly affected by such pollutants resulting from anthropogenic activities. The plant parts of interest for direct transfer of heavy metals in the human body are the edible parts such as the rice grains, which may consequently become a threat to human health. Nevertheless, heavy metals in the environment, consequently, are of immense concern, because of their persistence nature, bioaccumulation, and bio-magnification characters causing eco-toxicity in plants, animals, and human beings [11].

Elements such as arsenic (As), cadmium (Cd), lead (Pb) and mercury (Hg) have been identified as major risks to human health. Rice has been demonstrated to contain very high levels of As within its grain and is therefore potentially a major route of As exposure [12]. In Japan, where Cd contamination of arable lands due to mining activity and emissions from smelters has occurred, rice is the largest source of dietary intake of Cd, and thus been identified as a human health risk [13]. Also As, Cd, Pb and Hg have been identified as having a negative effect on plant growth [14].

On the other hand, the pollution of the arable lands by heavy metals, a side-effect of modern industry, has become increasingly severe. The levels of toxic minerals, especially cadmium (Cd) and lead (Pb), have been increasing in cereal grains, which threatens human health [15]. For example, Cd concentrations in soil samples collected in 2005 from Yangzhou, a developed area downstream of the Yangtze River in Jiangsu province, China, were significantly higher than those measured in the 1990s [16]. Moreover, in Taizhou, a developed area in Zhejiang province, the Cd and Pb concentrations in rice grains are approximately 8-fold and 4-fold greater, respectively, than those in other areas. Based on these data, the maximum daily intake of heavy metals by an adult in this area can reach 4.6-fold that of the Cd TDI (tolerable daily intake, according to FAO/WHO) and twice that of the Pb TDI due to the contamination in rice grains [17], beside other foods produced on the same lands that are consumed daily, such as vegetables.

Many researchers have reported trace element concentrations, especially for As, Cd, Pb and the other elements in rice grains from various countries. Rice cropped even from non-polluted areas may be contaminated because of using fertilisers with Cd and Pb [18,19].

Cultivated rice (*O. sativa*) is part of the *Oryza* genus, which is composed of 23 species, including the cultivated African rice *Oryza glaberrima*, plus 21 wild relatives [20]. These species show 11 different genome types (AA, BB, CC, BBCC, CCDD, EE, FF, GG, KKLL, HHJJ and HHKK), and have a pan-tropical distribution, growing in a broad range of environments [21]. Since *O. sativa* was domesticated from a limited number of *O. rufipogon* genotypes, its closest wild relative, it is estimated that only10-20% of the genetic diversity found in wild species are present in cultivated rice germplasm [22]. Although many efforts were made to find natural variation within the rice germplasm that could improve nutrition-related traits in cultivated rice, the narrow genetic diversity can be a limiting factor [23]. Considering the diversity of wild rice species and their distinct growing environments [21], we can expect that they will be adapted to different nutrient availabilities. Thus, wild relatives are a potential source of interesting alleles or even new mechanisms of metal and metalloid accumulation control. However, these genetic resources are almost unexplored, with very few studies screening these species for interesting phenotypes, especially for tolerance heavy metals-related traits.

Previous studies reported that a significant genotypic variation were detected in Cd, Cr, As, Ni and Pb concentrations in rice grains, indicating the possibility to reduce the concentrations of these heavy metals in grains through breeding approach [24]. There is a great difference among crop species and genotypes within a species in heavy metal uptake and accumulation [25]. The fundamental requirement for breeding low grain heavy metals accumulation rice cultivars is to know the genotypic variation in heavy metal accumulation and the physiological processes and genetic basis governing the heavy metal accumulation in rice grain [26]. Therefore, exploiting rice genotypes that do not accumulate heavy metals can be a viable option for rice cultivation in polluted areas or areas that oblige to irrigate with polluted water. Identification of these genotypes can also be a first step towards breeding rice genotypes that are highly tolerant to heavy metals [27].

Therefore, the present investigation was undertaken with two objectives. One is determining the concentrations of some heavy and essential elements in the seeds of the wild rice species (i.e., *Oryza rufipogon, Oryza officinalis* and *Oryza meyeriana*) and three rice cultivars belong to *Oryza sativa* specie. The other is estimating the genotypic variation between *Oryza officinalis* and its introgression lines in the accumulation of some elements to select the elite genotypes for rice improvement breeding programs for heavy metals tolerance.

2. MATERIALS AND METHODS

2.1 Plant Materials

Three wild rice species including *Oryza rufipogon*, *Oryza officinalis* and *Oryza meyeriana*, three rice cultivars belonging to *Oryza sativa* species including japonica type cultivar Nipponbare and two indicia type cultivars 93-11 and HY-8. Twenty-six introgression lines of *Oryza officinalis* as well as F_1 were used in this experiment.

2.2 The Site

The study was carried out at Biotechnology & Genetic Germplasm Institute, Yunnan Academy of Agricultural Sciences, Kunming, Yunnan, P.R. China.

2.3 Sample Analysis

The content of As, Pb, Rb, Sr, Ti and Zr (six heavy metal elements) and K, Ca, Fe, Mn, Zn, Cu and Mo (seven essential elements) were measured in the wild rice species and the three cultivated rice cultivars as well. Husk of 100 g grains has been removed after drying, and then ground into powder. The elements in each sample were measured by the handheld spectral analyzer (Thermo Niton XL3t 950). Three flag leaves of each rice cultivar in booting stage were selected and dried in the oven at 105°C for 30 minutes, and 80°C for 3 hours, and then ground them together into powder. Soil samples from the examined site were taken from the layer 0-20 cm mixed, air-dried and then ground together into powder.

Bioaccumulation Factor (BAF): The BAF (bioaccumulation factor, the ratio of the concentration of the element in the grains to that in the corresponding soil) was calculated for each rice sample to quantify the bioaccumulation effect of rice on the uptake of metal elements from the soil [28]. The BAF was computed as

$$BAF = Cr / Cs,$$

Where Cr and Cs represent the metal element concentrations in rice grains and soil, respectively.

2.4 Statistical Analysis

All the experiments were conducted in triplicate. To evaluate statistical differences in the elemental concentrations between rice types as well as between the parental genotypes and its introgression lines, the one-way ANOVA test was used. The SPSS statistical package version 15.0 was used for calculations (SPSS, version 15.0, Inc., Chicago, IL). Statistical significance was set at $p=0.05$.

The limit of detection (LOD) is an analytical threshold defining the smallest true value that can be distinguished from an analytical blank sample, with a specified probability of error [29,30]. Data sets containing values below the LOD are known as ‘censored data sets’ [31].

3. RESULTS AND DISCUSSION

3.1 Study of Elements

Heavy metal elements in rice grains: Several experimental and epidemiological studies have demonstrated that exposure to the heavy metal elements are associated with neurotoxic effects and motor function impairment [32], the cardiovascular system [33] and genetic damage [34]. In the present study, we have evaluated six heavy metal elements, i.e., As, Pb, Rb, Sr, Ti and Zr.

The mean concentrations of heavy metal elements in the grains of the three wild rice species and three rice cultivars were presented in Table 1. The results showed that the mean concentrations of the heavy metals in the soil were higher than that in the rice grains. The content ranking order of heavy metals in the paddy field soils was Ti > Zr > Sr > Pb > Rb > As.

Arsenic (As) levels in the rice grains from studied genotypes varied from 6.92 to 7.13 ppm, despite the non-statistical significance (Table 1). *Oryza meyeriana* presented the highest mean levels (7.13 ppm), on the other hand, HY-8 had the lowest concentration. Lead (Pb) concentrations in the rice grains varied from 6.56 to 6.98 ppm. *Oryza rufipogon* presented the highest mean levels (6.98 ppm), on the other hand, HY-8 had the lowest concentration. Rubidium (Rb) concentrations varied from 2.57 to 7.06 ppm. 93-11 presented the highest mean levels (7.06 ppm), and followed by *Oryza officinalis* (5.07 ppm). On the other hand, Nipponbare had the lowest concentration of Rb.

Strontium (Sr) levels in the rice grains varied from 7.02 to 16.36 ppm among genotypes. *Oryza meyeriana* presented the highest mean levels (16.36 ppm). On the other hand, HY-8 had the lowest concentration. Thallium (Ti) concentrations varied from 138.93 to 184.72 ppm. *Oryza meyeriana* presented the highest mean levels (184.72 ppm). On the other hand, Nipponbare had the lowest concentration. Zirconium (Zr) concentrations varied from 7.93 to 10.36 ppm. *Oryza meyeriana* presented the highest mean levels (10.36 ppm), on the other hand, HY-8 had the lowest concentration.

It is noticed that, when the concentrations of heavy metals in soils were high it would be high in rice plants too, such as As, Cr, Rb, Sr and Zr, thereby indicating that the element contents in rice plants were closely related to their availability in soils [35].

Evaluation of essential elements in rice grains: Essential elements are required by an organism to maintain its normal physiological function. Many of these elements are part of protein complexes (e.g. metalloproteins), which are required for enzymatic activities and can play structural roles in connective tissue or cell membranes.

The mean concentrations of the seven essential elements in the grains of the three wild rice species and three rice cultivars were shown in Table 2. The results demonstrated that the mean concentrations of the examined essential elements in the soil were mostly higher than that in the rice grains, except for potassium and molybdenum. The ranking order of these essential elements in the paddy field soils was Ca > Fe > K > Mn > Zn > Cu > Mo.

Potassium (K) concentrations in the rice grains from the all studied genotypes varied from 6131.63 to 9508.5 ppm (Table 2). HY-8 had the highest concentration (9508.5 ppm), on the other hand, *Oryza meyeriana* presented the lowest highest mean levels (6131.63 ppm). Calcium (Ca) mean concentrations in the rice grains varied from 608.41 to 2124.46. *Oryza meyeriana* presented the highest mean levels (2124.46 ppm), on the other hand, Nipponbare had the lowest concentration. These results are in agreement with those reported by Tisdale [36].

The mean concentrations of iron (Fe) varied from 44.94 to 46.22 ppm. *Oryza meyeriana* presented the highest mean levels (46.22 ppm). On the other hand, 93-11 had the lowest concentration. Rice is not an important source for iron compared to beans and meats for instance. This result is in a harmony with that obtained by Batista [37].

Manganese (Mn) levels in grains for the all studied genotypes varied from 55.97 to 57.6 ppm. Nipponbare presented the highest mean levels (57.6 ppm), followed by *Oryza officinalis* (57.57 ppm) and on the other hand, *Oryza meyeriana* had the lowest concentration. Zinc (Zn), the mean concentrations varied from 14.49 to 43.75 ppm. *Oryza officinalis* presented the highest mean levels (43.75 ppm), on the other hand, Nipponbare had the lowest concentration. Zn plays an important role in the metabolism at process in both plants and animals, an excessive zinc maybe toxic to organisms. This result is in agreement with those reported by Wan [38].

The mean concentrations of copper (Cu) varied from 16.90 to 18.72 ppm. *Oryza officinalis* presented the highest mean levels (18.72 ppm), on the other hand, 93-11 had the lowest concentration. Molybdenum functions as an enzyme cofactor. Molybdenum-enzymes catalyse the hydroxylation of various substrates. Molybdate might also be involved in stabilising the steroid-binding ability of unoccupied steroid receptors [39]. The mean concentrations of molybdenum (Mo) in grains for the all studied genotypes varied from 3.80 to 5.01 ppm.

The micronutrients include seven essential elements, which are boron, copper, chlorine, iron, manganese, molybdenum, and zinc. These elements occur in very small amounts in both soil and plants, but their role is as important as the primary nutrients [40]. A deficiency of one or more of the micronutrients can lead to severe depression in growth, yield and crop quality.

Bioaccumulation Factor (BAF): Bioaccumulation factors (BAFs) for the metallic elements transfer from soils to rice are shown in Table 3. The BAF values of the metal elements such as As, Pb, Rb, Sr, Ti, Zr, Mn, Zn and Cu varied from 0.02 for Ti to 0.84 for As. The trend in the BAF for metal elements in the study site was in the ranking order of As > Cu > Zn > Pb > Sr >Rb > Mn> Zr > Ti. BAF > 1 indicates that plant accumulates more heavy metals than soil does. BAF values of all metal elements in this investigation were less than one for rice grains, which indicates poor accumulation of heavy metals in grains. These results are in partial agreement with those reported by [41], probably due to the differences in examined sites between two studies.

3.2 Study of Elements in the Parental Genotypes and Its Introgression Lines

3.2.1 Evaluation of heavy metal elements in rice leaves

Analysis of variance of heavy metals: Significant differences were exhibited among the parental rice genotypes and its introgression lines for the studied traits as shown in Table 4. The data illustrate the presence of high amount of genetic variability among these genotypes.

Table 1. Mean concentrations (± SD) of some heavy metals in the grains of rice genotypes

Genotypes	As (ppm)	Pb (ppm)	Rb (ppm)	Sr (ppm)	Ti (ppm)	Zr (ppm)
Nipponbare	< LOD±2.03	< LOD±2.74	1.59±0.96	8.46 ±0.97	< LOD±78.93	7.58±1.27
93-11	< LOD±1.99	< LOD±2.69	6.10±0.96	9.25 ±0.96	78.75 ±71.38	6.95 ±1.22
HY-8	< LOD±1.92	< LOD±2.56	1.86±0.82	6.31±0.89	< LOD±81.11	6.62±1.21
Oryza rufipogon	< LOD±2.09	< LOD±2.98	3.98±0.93	12.81 ±1.07	85.48 ±68.65	8.18 ±1.29
Oryza officinalis	< LOD±2.09	< LOD±2.84	4.16±0.91	12.09 ±1.03	< LOD±79.87	7.81±1.26
Oryza meyeriana	< LOD±2.13	< LOD±2.90	3.87±0.91	15.25 ±1.11	110.79 ±73.93	9.07±1.29
Soil	< LOD±3.38	12.54 ±2.89	13.42 ±1.36	28.60 ±1.53	7668.65±198.13	60.49 ±2.23
LOD	5.0	4.0	6.0	8.0	60.0	3.0

Table 2. Mean concentrations (± SD) of some essential elements in the grains from different rice genotypes

Genotypes	K (ppm)	Ca (ppm)	Fe (ppm)	Mn (ppm)	Zn (ppm)	Cu (ppm)	Mo (ppm)
Nipponbare	7626.02±303.37	562.42 ±79.09	< LOD±21.18	< LOD±27.60	10.99 ±3.50	<LOD±8.34	3.35 ±1.58
93-11	8893.64±326.39	548.05 ±60.36	< LOD±19.94	< LOD±26.10	12.26 ±3.43	< LOD±7.90	2.75±1.78
HY-8	9174.23±334.27	700.66 ±88.11	< LOD±20.04	< LOD±26.33	15.28 ±3.57	< LOD±7.91	2.78±1.54
Oryza rufipogon	8202.56±309.40	1008.01 ±91.72	< LOD±20.79	< LOD±27.08	30.59 ±4.22	< LOD±8.25	2.91±2.10
Oryza officinalis	7319.76±302.98	1150.26 ±95.88	< LOD±20.46	< LOD±27.57	39.35 ±4.40	11.35 ±7.37	<LOD±2.30
Oryza meyeriana	5857.28±274.35	2012.30 ±112.16	< LOD±21.22	< LOD±25.97	24.18 ±3.93	< LOD±8.28	< LOD±2.32
Soil	3860.79±266.54	12055.26±283.36	10517.95±111.88	260.19 ±33.59	40.91 ±5.11	22.75 ±7.08	< LOD±2.58
LOD	250.0	70.0	25.0	30.0	6.0	9.0	1.5

Table 3. Bioaccumulation factor of some metal elements in this study

Genotypes	As	Pb	Rb	Sr	Ti	Zr	Mn	Zn	Cu
Nipponbare	0.84	0.44	0.17	0.31	0.018	0.14	0.196	0.32	0.58
93-11	0.83	0.43	0.48	0.34	0.019	0.13	0.191	0.34	0.57
HY-8	0.83	0.43	0.18	0.24	0.018	0.12	0.192	0.41	0.57
Oryza rufipogon	0.85	0.45	0.33	0.46	0.020	0.15	0.194	0.76	0.58
Oryza officinalis	0.85	0.44	0.34	0.44	0.018	0.15	0.196	0.95	0.63
Oryza meyeriana	0.85	0.45	0.32	0.54	0.023	0.17	0.191	0.61	0.58
Mean	0.84	0.44	0.30	0.39	0.02	0.14	0.19	0.57	0.59

Table 4. Analysis of variance and mean squares of some heavy metals in the leaves of parental genotypes and its introgression lines

S.O.V	d.f	As	Pb	Rb	Sr	Ti	Zr
Rep	2	0.74	9.94	5.19	23.77	54.7	31.7
Genotypes	28	95.67**	11.77**	2404.23**	95.04**	321.11*	303.44**
Error	56	0.01	0.01	1.78	0.12	3.17	0.13

***: significant at 0.01 probability level*

3.2.2 Mean concentrations of heavy metal elements in rice leaves

The mean concentrations of heavy metal elements in the leaves of parental genotypes and 26 introgression lines were illustrated in Table 5. The results showed that the mean concentrations of the heavy metals in the soil were higher than that in the rice leaves.

Arsenic (As) mean concentrations in the rice leaves for all studied genotypes ranged from 5.19 to 12.97 ppm (Table 5), FC7-12 presented the highest mean concentrations (12.97 ppm), followed by lines FC7-6, FC7-20 and FC7-1 respectively. On the other hand, FC7-14 had the lowest concentration. *Oryza officinalis* (Parent 1) had 6.79 ppm and HY-8 (Parent 2) had 6.49 ppm. Four introgression lines exceeded both parental genotypes while the remaining lines had similar concentrations with both of parental genotypes. Lead (Pb) mean concentrations in leaves ranged from 4.06 to 11.91 ppm. Line FC7-12 presented the highest mean concentrations (11.91 ppm), followed by 20, while, FC7-21 had the lowest concentration. Only two lines exceeded the highest parent and the remaining lines were closed with both of the parents.

The mean concentrations of rubidium (Rb) varied from 7.88 to 12.56 ppm. Line FC7-12 presented the highest mean concentrations (12.56 ppm), while, F_1 had the lowest concentration. All the lines exceeded positively both parental genotypes. Strontium (Sr) levels in the leaf for the all studied genotypes varied from 21.28 to 36.95 ppm. FC7-12 presented the highest mean concentrations (12.56 ppm), meanwhile, F_1 had the lowest concentration. All the lines exceeded positively both parental genotypes.

The mean concentrations of thallium (Ti) varied from 64.01 to 106.98 ppm. Line FC7-12 presented the highest mean concentrations (106.98 ppm), while, FC7-23 had the lowest concentration. No introgression line exceeded the highest parental genotype (*Oryza officinalis*) except line FC7-12, most of lines very close with the lower parental genotype (HY-8). Zirconium (Zr), the mean concentrations varied from 4.18 to 8.71 ppm. The two parental genotypes showed closed Ti concentrations. Out of 26 introgression lines, nine exceeded both parental genotypes while the remaining lines had similar concentrations to those of both of parental genotype.

Concentrations of heavy metals vary in different parts of rice plants, and higher concentrations occur in the leaves comparing to seeds [42]. The results in this study are in a harmonious agreement with those reported in previously mentioned study [42].

3.2.3 Evaluation of the essential elements in rice leaves

Analysis of variance of essential metals: Significant differences were exhibited among the parental genotypes and its introgression lines for the studied traits as shown in Table 6. The data illustrate the presence of high amount of genetic variability among these genotypes.

Table 5. Mean concentrations (± SD) of some heavy metals in the leaves of parental genotypes and its introgression lines

Genotypes	As			Pb			Rb			Sr			Ti			Zr		
Soil	< LOD	±	1.38	12.54	±	2.89	13.42	±	1.36	38.6	±	1.83	7668.65	±	19.13	60.49	±	2.23
Oryza officinalis	5.5	±	1.29	< LOD	±	1.16	6.93	±	0.87	10.34	±	1.05	87.93	±	5.61	6.16	±	0.19
HY-8	< LOD	±	1.49	< LOD	±	0.86	< LOD	±	0.73	13.44	±	1.14	< LOD	±	4.31	6.18	±	0.33
F1	< LOD	±	1.98	< LOD	±	0.83	7.02	±	0.86	25.862	±	1.34	< LOD	±	4.62	5.754	±	0.31
FC7-1	6.73	±	2.66	< LOD	±	0.73	8.36	±	0.95	32.16	±	1.57	< LOD	±	4.52	4.99	±	0.41
FC7-3	< LOD	±	1.41	< LOD	±	0.15	8.18	±	0.91	23.38	±	1.36	< LOD	±	4.04	6.53	±	0.37
FC7-4	< LOD	±	1.2	< LOD	±	0.71	8.33	±	0.85	25.77	±	1.34	< LOD	±	4.94	3.91	±	0.27
FC7-5	< LOD	±	1.34	< LOD	±	0.66	8.46	±	0.88	23.52	±	1.31	< LOD	±	4.31	7.35	±	0.33
FC7-6	8.79	±	2.64	< LOD	±	0.8	7.95	±	0.85	26.84	±	1.36	< LOD	±	4.25	5.46	±	0.3
FC7-7	< LOD	±	1.46	< LOD	±	0.81	9.03	±	0.96	23.28	±	1.38	< LOD	±	4.02	8.29	±	0.42
FC7-7	< LOD	±	1.19	< LOD	±	0.75	8.69	±	0.89	22.51	±	1.29	< LOD	±	4.24	4.92	±	0.29
FC7-8	< LOD	±	1.25	< LOD	±	0.81	7.79	±	0.83	24.61	±	1.31	< LOD	±	4.29	5	±	0.28
FC7-9	< LOD	±	1.24	< LOD	±	0.87	7.53	±	0.86	21.64	±	1.28	< LOD	±	4.87	5.23	±	0.3
FC7-10	< LOD	±	1.24	< LOD	±	0.88	8.47	±	0.86	25.8	±	1.34	< LOD	±	4.54	5.87	±	0.3
FC7-11	< LOD	±	1.26	< LOD	±	0.9	8.32	±	0.87	23.78	±	1.31	< LOD	±	4.88	6.84	±	0.32
FC7-12	10.15	±	2.82	9.88	±	2.03	11.39	±	1.17	34.58	±	2.37	101.41	±	5.57	6.53	±	0.15
FC7-13	< LOD	±	1.36	< LOD	±	1.11	10.74	±	0.98	25.56	±	1.37	< LOD	±	4.8	7	±	0.35
FC7-14	< LOD	±	1.14	< LOD	±	0.77	10.41	±	0.94	34.3	±	1.49	< LOD	±	4.59	5.55	±	0.33
FC7-15	< LOD	±	1.27	< LOD	±	0.88	9.31	±	0.92	30.13	±	1.43	< LOD	±	4.38	6.53	±	0.34
FC7-16	< LOD	±	1.42	< LOD	±	1.01	8.28	±	0.9	22.74	±	1.35	< LOD	±	4.82	5.91	±	0.36
FC7-17	< LOD	±	1.23	< LOD	±	0.75	8.11	±	0.88	26.1	±	1.36	< LOD	±	4.46	4.09	±	0.29
FC7-18	< LOD	±	1.29	< LOD	±	0.88	8.01	±	0.88	26.97	±	1.37	< LOD	±	4.47	6.89	±	0.33
FC7-19	< LOD	±	1.32	< LOD	±	0.91	8.16	±	0.88	27.56	±	1.4	< LOD	±	4.86	5.06	±	0.32
FC7-20	6.89	±	1.74	< LOD	±	1.21	8.26	±	0.88	29.79	±	1.44	< LOD	±	4.89	6.07	±	0.35
FC7-21	< LOD	±	1.33	< LOD	±	0.06	8.38	±	0.9	22.75	±	1.31	< LOD	±	4.5	5.66	±	0.32
FC7-22	< LOD	±	1.21	< LOD	±	0.91	7.79	±	0.86	22.98	±	1.3	< LOD	±	4.12	5.2	±	0.3
FC7-23	< LOD	±	1.2	< LOD	±	0.76	8.46	±	0.88	24.66	±	1.33	< LOD	±	4.01	6.1	±	0.32
FC7-24	< LOD	±	1.22	< LOD	±	0.91	8.03	±	0.86	20.04	±	1.24	< LOD	±	4.46	5.45	±	0.29
FC7-25	< LOD	±	1.19	< LOD	±	0.72	8.39	±	0.88	27.81	±	1.39	< LOD	±	4.29	3.98	±	0.29
FC7-26	< LOD	±	1.2	< LOD	±	0.88	8.81	±	0.93	22.52	±	1.28	82.35	±	4.9	7.7	±	0.32
LOD	5			4			6			8			60			3		

Table 6. Analysis of variance and mean squares of some essential elements in the leaves of parental genotypes and its introgression lines

S.O.V	d.f	K	Ca	Fe	Mn	Zn	Cu	Mo
Rep	2	2421.84	18320.15	1.35	63.2	6.32	15.97	4.9
Genotypes	28	12519924**	9763295**	573.71**	690.95**	545.31**	102.17**	8.48**
Error	56	19147.78	6514.45	0.13	0.57	0.03	0.16	0.01

***: significant at 0.01 probability level*

3.2.4 Mean concentrations of essential elements in rice leaves

The mean concentrations of the essential elements in the leaves of parental genotypes and 26 introgression lines were shown in Table 7. The results revealed that the mean concentrations of the essential elements in soil were higher than that in rice leaves. Plants absorb the essential elements through their root systems or their leaves in various forms.

Potassium (K) mean concentrations in rice leaves for the all studied genotypes varied from 12822.63 to 28037.9 ppm (Table 7). Line FC7-26 presented the highest mean concentrations of potassium (28037.9 ppm), followed by FC7-12 and FC7-7 respectively. On the other hand, FC7-6 had the lowest concentration. *Oryza officinalis* (parent 1) had 19441.73 ppm and HY-8 (parent 2) had 12122.7 ppm. Nine introgression lines exceeded the higher parental genotypes while the remaining lines had exceeded the lower ones. Calcium (Ca) mean concentrations in leaf ranged from 9865.11 to 18884.86 ppm. Line FC7-14 presented the highest mean concentrations (18884.86 ppm), followed by lines FC7-20 and FC7-1, respectively. Meanwhile, line FC7-24 had the lowest concentration. Apparently, all the studied rice genotypes exceeded both of parental genotypes.

The mean concentrations of iron (Fe) varied from 27.08 to 69.01 ppm. Line FC7-7 had the highest mean concentration (69.01 ppm), followed by lines FC7-12, FC7-15 and FC7-13, respectively. Meanwhile, F_1 had the lowest concentration. It was considerable interest to note that a majority of the introgression lines exceeded positively both of parental genotypes.

Manganese (Mn) levels in leaf for the all studied genotypes varied from 32.09 to 105.97 ppm. Line FC7-10 had the highest mean concentrations (105.97 ppm), followed by lines FC7-12 (94.77 ppm) and line FC7-14 (67.81 ppm), respectively. Meanwhile, line FC7-1 had the lowest concentration. Nine introgression lines exceeded the higher parental genotypes while the remaining lines had exceeded positively lower. The mean concentrations of zinc (Zn) varied from 6.23 to 14.70 ppm. Line FC7-12 had the highest mean concentration (16.70 ppm, while, line FC7-11 had the lowest concentration. Eight introgression lines exceeded the higher parental genotypes (*Oryza officinalis*), while the remaining lines had exceeded positively the lower ones.

In general, soil contains large amounts of all elements, but only a very small percentage of these total amounts is actually useful for plant growth [43]. This is noticed in the most essential elements that were examined in this study, particularly in case of iron concentration exceeding 10,000 ppm while the concentration found in leaves was less than 75 ppm. The results were found to be in agreement with those previously reported by Hodges [44].

The mean concentrations of copper (Cu) varied from 13.01 to 23.27 ppm. Line FC7-12 had the highest mean concentration (23.27 ppm), while, line FC7-8 had the lowest concentration. No introgression line exceeded the highest parental genotype (HY-8) except for line FC7-12. Most of the lines had Cu concentrations similar to the lower parental genotype (*Oryza officinalis*). The mean concentrations of molybdenum (Mo) in the rice leaves for all the studied introgression lines that varied from 3.29 to 7.55 ppm. The plants exhibited for Mo contents the same as Cu concentrations. No introgression lines exceeded the highest parental genotype except line FC7-12, while the remaining lines had concentrations of Mo similar to the lower parental genotypes.

Most essential metals; Fe, Mn, Zn, Mo and Cu that were found profusely in the paddy plants where the micronutrients that are required for various enzyme activities and play important roles in photosynthesis and growth of the plant [45].

3.2.5 Estimation of genetic parameters for the concentrations of examined heavy and essential elements in parental genotypes and its introgression lines

Considerable improvement and successful breeding programs for any crop largely depends on the amount of genetic variability among genotypes, which selected further manipulation to achieve the breeding target. A survey of genetic variability with the help of suitable parameters such as genotypic (σ^2g) and phenotypic (σ^2p) variance, genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), heritability ($h^2b.s$) in broad sense and genetic advance (GA) is necessary to start an efficient breeding program.

A wide range of variations in the accumulation of ten measured elements was observed between parental genotypes and its introgression lines (Table 8).

Table 8 presents estimates of genotypic (σ^2g) and phenotypic (σ^2p) variance, genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), heritability ($h^2b.s$) in broad sense and genetic advance (GA) of parental genotypes and its 26 introgression lines. Expectedly, phenotypic variance was generally higher than the genotypic variance in all the measured elements.

Table 7. Mean concentrations (± SD) of some essential metals in the leaves of parental genotypes and its introgression lines

Genotypes	K (ppm)			Ca (ppm)			Fe (ppm)			Mn (ppm)			Zn (ppm)			Cu (ppm)			Mo (ppm)		
Soil	3860.8	±	266.5	12055.3	±	283.4	10518.0	±	111.9	260.19	±	13.59	40.91	±	5.11	22.75	±	2.08	< LOD	±	0.58
Oryza officinalis	19441.7	±	415.5	3607.0	±	144.6	28.00	±	2.65	32.00	±	3.00	8.00	±	0.51	< LOD	±	1.54	4.15	±	0.65
HY-8	12122.7	±	333.2	6430.9	±	172.1	< LOD	±	2.25	< LOD	±	2.02	< LOD	±	0.33	14.00	±	1.7	< LOD	±	0.44
F1	18820.4	±	387.3	13334.8	±	230.7	< LOD	±	2.681	< LOD	±	2.332	< LOD	±	0.29	< LOD	±	1.12	< LOD	±	0.35
FC7-1	17534.0	±	380.0	18277.0	±	268.9	< LOD	±	2.08	< LOD	±	2.09	< LOD	±	0.29	< LOD	±	1.33	< LOD	±	0.5
FC7-3	16974.8	±	376.1	12652.7	±	228.5	< LOD	±	2.73	< LOD	±	2.44	8.38	±	0.45	< LOD	±	1.08	< LOD	±	0.43
FC7-4	15066.2	±	356.0	14778.5	±	244.0	< LOD	±	2.42	48.51	±	3.94	8.24	±	0.38	< LOD	±	1.31	< LOD	±	0.32
FC7-5	17447.0	±	379.3	12341.7	±	225.2	28.93	±	3.13	38.12	±	3.09	9.28	±	0.48	< LOD	±	1.31	< LOD	±	0.33
FC7-6	12822.6	±	324.8	15365.7	±	242.4	< LOD	±	2.94	< LOD	±	2.17	< LOD	±	0.37	< LOD	±	1.24	< LOD	±	0.32
FC7-7	25637.8	±	485.4	12674.5	±	241.5	61.27	±	7.74	< LOD	±	2.82	< LOD	±	0.32	< LOD	±	1.01	< LOD	±	0.48
FC7-7	15765.3	±	351.3	11039.3	±	207.6	< LOD	±	2.69	< LOD	±	2.21	< LOD	±	0.24	< LOD	±	1.08	< LOD	±	0.35
FC7-8	17675.2	±	377.2	15291.5	±	245.5	< LOD	±	2.17	< LOD	±	2.76	8.16	±	0.34	< LOD	±	1.01	< LOD	±	0.29
FC7-9	13634.5	±	329.7	12286.0	±	217.2	< LOD	±	2.09	38.63	±	3.14	< LOD	±	0.37	< LOD	±	1.36	< LOD	±	0.36
FC7-10	15487.9	±	351.3	16301.7	±	249.1	< LOD	±	2.6	100.9	±	5.07	< LOD	±	0.33	< LOD	±	1.14	< LOD	±	0.31
FC7-11	14898.4	±	345.5	14772.1	±	238.0	< LOD	±	2.55	< LOD	±	2.22	< LOD	±	0.23	< LOD	±	1.32	< LOD	±	0.34
FC7-12	26053.4	±	503.5	10303.3	±	198.9	44.78	±	3.86	89.81	±	4.96	15.05	±	1.65	21.26	±	2.01	6.76	±	0.79
FC7-13	22706.3	±	489.1	11357.8	±	223.4	36.13	±	3.03	< LOD	±	2.67	8.42	±	0.31	< LOD	±	1.17	< LOD	±	0.38
FC7-14	23466.4	±	447.5	18884.9	±	279.1	26.08	±	2.85	63.04	±	4.77	8.9	±	0.36	< LOD	±	1.37	< LOD	±	0.35
FC7-15	23236.9	±	449.6	14544.0	±	254.8	38.69	±	3.41	< LOD	±	2.92	11.08	±	0.45	< LOD	±	1.16	< LOD	±	0.36
FC7-16	19561.8	±	400.1	14298.6	±	242.0	< LOD	±	2.24	< LOD	±	2.21	8.28	±	0.33	< LOD	±	1.18	< LOD	±	0.47
FC7-17	16572.7	±	351.9	14455.0	±	230.3	< LOD	±	2.65	58.21	±	4.74	< LOD	±	0.28	< LOD	±	1.19	< LOD	±	0.36
FC7-18	17612.7	±	382.7	16008.5	±	254.7	< LOD	±	2.54	< LOD	±	2.99	< LOD	±	0.3	< LOD	±	1.24	< LOD	±	0.36
FC7-19	22423.0	±	473.4	13466.7	±	246.4	< LOD	±	2.77	< LOD	±	2.66	12.49	±	0.93	< LOD	±	1.26	< LOD	±	0.4
FC7-20	19321.0	±	403.8	18307.3	±	274.3	< LOD	±	2.85	< LOD	±	2.44	8.56	±	0.45	< LOD	±	1.26	< LOD	±	0.35
FC7-21	17859.2	±	372.9	11811.1	±	215.8	34.91	±	2.4	< LOD	±	2.11	7.46	±	0.37	< LOD	±	1.428	< LOD	±	0.36
FC7-22	20161.7	±	410.0	13722.5	±	240.6	< LOD	±	2.09	< LOD	±	2.73	8.78	±	0.42	< LOD	±	1.24	< LOD	±	0.36
FC7-23	13578.1	±	326.8	14000.9	±	228.7	< LOD	±	2.33	61.02	±	3.8	< LOD	±	0.37	< LOD	±	1.2	< LOD	±	0.36
FC7-24	17300.8	±	361.9	9865.1	±	196.2	< LOD	±	2.33	36.21	±	2.9	< LOD	±	0.32	< LOD	±	1.38	< LOD	±	0.35
FC7-25	18783.4	±	396.3	14440.3	±	245.1	< LOD	±	2.83	< LOD	±	2.36	< LOD	±	0.24	< LOD	±	1.11	< LOD	±	0.35
FC7-26	28037.9	±	541.4	10609.2	±	233.4	< LOD	±	2.7	< LOD	±	2.87	9.69	±	0.46	< LOD	±	1.12	< LOD	±	0.32
LOD	250			70			25			30			6			12			3		

Table 8. Estimation of genetic parameters for some heavy and essential elements in different rice genotypes

Elements	G.V	P.V	G.C.V	P.C.V	Hbs %	GA	GA%
As	0.3	0.46	13.66	16.92	63.23	0.91	22.73
Pb	3.92	3.95	25.42	25.42	97.95	4.08	52.36
Rb	5.04	5.09	55.38	55.65	97.01	4.6	113.51
Sr	31.64	31.76	21.21	21.26	97.61	11.56	43.62
Ti	105.98	109.15	7.54	7.65	95.1	20.9	15.31
Zr	2.566	2.803	19.68	20.57	89.56	3.16	38.8
Fe	189.96	190.88	33.53	33.61	97.52	28.32	68.91
Mn	230.13	230.7	25.95	25.98	97.75	31.21	53.38
Zn	12.53	12.68	36.72	36.93	96.86	7.25	75.22
Cu	1.26	1.65	10.93	12.47	74.79	2.03	19.73

High genotypic and phenotypic variances were observed in Mn concentration (230.13 and 230.7) followed by Fe concentration (189.96 and 190.88), respectively. The highest estimated value of genotypic (GCV) and phenotypic coefficients of variation (PCV) were recorded for Rb concentrations, followed by Zn and Fe concentrations, with estimated values of (55.38 and 55.65), (36.72 and 36.93) and (33.53 and 33.61), respectively. Moreover, the phenotypic coefficient of variation (PCV) was close to the genotypic coefficient of variation (GCV) for all the ten measured elements. Furthermore, high estimates of broad sense heritability (h^2b.s) were exhibited for all measured elements; it was ranged from 63.23% for As concentration to 97.95% for Rb concentration. These findings indicated that the concentrations of most of measured elements are mainly controlled by genetic factors. Thus, the breeding methods could be efficiently used in order to improve and obtain desired accumulation of these elements in rice. These results are in conformity with findings made by El-Habet [46].

In addition, the results revealed that the genetic advance was high for Mn concentration (31.21) followed by Fe concentration (28.32) and Ti concentration (20.90), while, the lowest value was detected for As concentration (0.91). The genetic advance is an efficient evidence of the progress that is predicted as a result of the related choice population. Heritability in conjunction with genetic advance would give a more reliable index of better selection. High heritability compared with high genetic advance were recorded for Mn, Fe and Ti concentrations, suggesting the effectiveness of selection in early generation to improve the ability of accumulation of these elements in rice in desired way. These results are in partial agreement with that recorded earlier by Yan [26].

4. CONCLUSION

From the obtained data of this study, it can be concluded that the mean concentrations of the elements in the soil were higher than in the rice seeds for all the studied rice genotypes, except for potassium and molybdenum. Bioaccumulation Factor (BAF) values of all elements were less than one in the rice seeds which indicates that metals absorbed by plants are poorly accumulated in grains. Element concentrations in leaves were much higher than that in seeds. The great diversity among the rice genotypes may help to breed new varieties with lower heavy metal accumulation and increased accumulation of essential elements. The introgression lines FC7-12, FC7-6, FC7-20 and FC7-1 showed a good performance, achieving low accumulation of heavy metals. Finally, these selected introgression lines could be used as donors for future breeding programs that aim to develop rice varieties with low heavy metal accumulation.

ACKNOWLEDGEMENT

This work was supported by the Ministry of Science and Technology Project (2017YFD0100202), Major Plan Projects of Science and Technology in Yunnan (2018ZG005), National Science Fund Project (31460054), Plan Projects of Science and Technology in Yunnan (2018FB035), Agricultural Joint Key Project in Yunnan [2017FG001(-007)], Key Projects of Applied Basic Research in Yunnan (2015FA033), China.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history/27541