IDENTIFICATION OF RICE GENOTYPES RESPONSIBLE FOR HIGH-EFFICIENT AND LOW-EFFICIENT MICRONUTRIENTS CONTENTS

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ABSTRACT
Micronutrient raises to the relative quantity of a nutrient that is prerequisite for plant growth. It takes part in metabolic activities, enzymatic process/catalysts and these all directly and in-directly support in plant growth development and essential to alive organisms for normal growth and reproduction. The incidence of micronutrient deficiency has increased in recent year. An experiment was undertaken to investigate the important micronutrients under laboratory conditions in the Plant Nutrition, College of Resources, Sichuan Agricultural University, Chengdu, Sichuan Provine of China. A total of seventy (70) different commercial rice genotypes were tested to assess Zinc (Zn), Iron (Fe), Copper (Cu) Manganese (Mn) and Selenium (Se) contents in rice grain. These contents were determined by using Optical Emission Spectrometry (OES) with Acid Digestion method. The samples were digested by using the application of acid mixture which includes nitric acid (HNO_3): and perchloric acid ($HClO_4$). Results revealed that four Mn and Cu, three Fe, eight Zn and two Se rice genotypes were high contents in rice grain. However, the lowest grain contents were recorded in two genotypes in Mn, three genotypes in Fe, Cu and Zn and two genotypes in Se. The highest Zn content 37.73 mg kg^{-1} was documented in ZJ-14 genotype and lowest 18.28 mg kg^{-1} in ZJ-29. The highest Fe 23.73 mg kg^{-1} content was found in YR-5 genotype and lowest 8.68 mg kg^{-1} in ZJ-25. However, the highest Cu content 4.99 mg kg^{-1} was found in ZJ-11 genotype and lowest 1.34 mg kg^{-1} in ZJ-26. In case of the highest Mn content 54.16 mg kg^{-1} was recorded in ZJ-17 mg kg^{-1} genotype and lowest 17.94 mg kg^{-1} in ZJ-28. The ZJ-22 genotype was revealed the highest Se content 0.56 mg kg^{-1} and YR-30 was found in lowest contents 0.04 mg kg^{-1} of Se. Hence, from the results it shows the variation among the selected commercial rice genotypes for micronutrients status in grains and indicated the different behavior of the genotypes for the uptake of micronutrients.
Key words: Genotypes, nutrient deficiency, rice grain, uptake, yield.

INTRODUCTION
Rice (*Oryza sativa* L.) is the most essential staple food consumed by more than half of the world's population and more than 3.5 billion people depend in the world (Joy *et al.,* 2015; Gadal *et al.,* 2019). Unfortified modern rice varieties with small amounts of micronutrients in the grains supply only a fraction of the daily individual requirements. Dependence on rice as the major dietary source of micronutrients contributes to micronutrient deficiency (Palanog *et al.,* 2019). China is the most populous country in the world and consumes rice 143.8 million metric tons per annum (Statista, 2020). Production of rice has been tripled over the past several decades (Huang *et al.,* 2018). These advances in rice production throughout the world are paramount importance for food security (Peng *et al.,* 2015). However, considerable genotypic differences have been documented in the

concentration of micronutrients such as Zinc, Iron, Copper Manganese and Selenium in rice grains (Peng *et al.*, 2015).

Micronutrients, Zn and Fe specifically plays pivotal role in the growth and development, of human beings (Shenkin, 2006). The Recommended Dietary Allowance (RDA) of Zn and Fe are respectively 15 mg and 10 mg per day for man. However, micronutrient deficiencies affect more than half of the world's population, especially women and preschool children. Zinc deficiency is also considered to be quite common and affects newborn, children, pregnant women and old people (Joy *et al.*, 2015). Rice is one of highly sensitive crops to Zn deficiency and Zn limits growth and yield of rice. Zinc deficiency in rice has been widely reported in many rice-growing regions of the world. Increasing Zn concentration of rice grains, and bioavailability of food crop, through bio-fortification appears to be the most feasible, sustainable and economical approach among the different interventions to address human Zn deficiency (Salunke *et al.*, 2011).

According to a report by the World Health Organization in 2002, Fe deficiency affects over 3 billion people in the world, especially in developing countries. Iron deficiency causes impairments in mental and psychomotor development in children and diminished productivity in adults, and represents the most common cause of anemia (Bashir *et al.*, 2019) however, in biological systems and is receiving growing concern worldwide because of increasing reports about Fe deficiencies in humans. Inadequate intake with poor bioavailability of Fe in foods is in general the main cause of global Fe deficiency in humans. Thus, improved Fe concentration as well as bioavailability in rice will generate major health benefits for a large number of susceptible people. In response to this problem, many potential approaches have been proposed and applied to increase bioavailable Fe concentrations in rice endosperm. High concentrations of trace metals, including Cu may pose significant influences on the quality of rice, and consequently affect human health (Zhu *et al.*, 2009; Adrees *et al.*, 2015; Lu *et al.*, 2015).

Among trace metals, the redox-active element Cu is an important nutrient for plant growth, however, high concentration of Cu in soil shows highly toxic effect to plant, such as growth inhibition and oxidative damage by generating reactive oxygen radicals (Yruela, 2009; Wu *et al.*, 2010; Thounaojam *et al.*, 2012; Adrees *et al.*, 2015). Manganese is an essential trace element for plants, domestic animals, and humans (Underwood, 1987). In mammals, Mn is necessary for proper fetal development and growth and is crucial throughout the life span (Wood, 2009; Zota *et al.*, 2009). Humans maintain stable tissue levels of Mn and Mn is present in virtually all diets at low concentrations (Aschner *et al.*, 2005). The U.S. National Research Council has established an estimated safe and adequate dietary Mn intake of 2-5 mg per day for adults (Greger, 1998). Overt Mn deficiency diseases are extremely rare, but segments of populations from remote areas still suffer from subclinical Mn deficiency (Underwood, 1987; Bjorklund *et al.*, 2017).

Selenium deficiency is another serious problem which causes of some human diseases, including Keshan disease and Kaschin-Beck disease in China (Li *et al.*, 2013). Selenium is considered as an essential micronutrient for animals and humans (Terry *et al.*, 2000; Sun *et al.*, 2010), and has an important role in improving the immune system and reducing the risk of cancer (Li *et al.*, 2008; Zhu *et al.*, 2009). The recommended level of Se recommended by the World Health Organization (WHO) for adults is 40 μg day^{-1} (Combs, 2001; Abdulah *et al.*, 2005; Sun *et al.*, 2010). However, in many regions in China, the daily Se intake of people is lower than the suggested level (Sun *et al.*, 2010), and its levels in some Se-deficient areas are less than 10 μg day^{-1} (Chen *et al.*, 2002). People could acquire Se from their diet to reduce the occurrence of diseases related to Se deficiency, as previously reported (Navarro-Alarcon 2008; Sun *et al.*, 2010). Se concentration in rice varies according to Se status of the paddy soil. The rice grown in soil with Se concentration within the range of 0.5 to 47.7 mg kg^{-1} could produce Se in wholegrain ranged from 0.084 to 9.67 mg kg^{-1} (Sun *et al.*, 2010).

This study will help in identifying the high efficient and low efficient micronutrients contents in rice genotypes among the existing Sichuan Provine of China genotypes and developing nutritionally improved rice cultivar and molecular breeding techniques along with the development of rice varieties with enhanced nutritional quality.

Materials and Methods

Experimental site

This experiment was analyzed at the experimental laboratory of Plant Nutrition, College of Resources, Sichuan Agricultural University, Chengdu, Sichuan Provine of China, during March 2019.

Experimental materials

In the experiment seventy different commercial rice genotypes were tested to assess Zinc, Iron, Copper, Manganese and Selenium high and low contents in rice grain. These contents were determined by using Optical Emission Spectrometry (OES) with Acid Digestion method.

Processing of seeds

Processing of seeds of seventy rice genotypes for chemical analysis were done by dehusking, husk from the rice grains was removed by using a laboratory de-husker (OHYA-25, Japan). From each genotypes 0.50 g of the rice seeds was weighed separately (Jahan *et al.,* 2013).

Determination of micronutrients content from the rice grain sample

Micronutrients content of rice grain samples were digested with HNO_3–H_2O_2 in a microwave accelerated reaction system (CEM, Matthews, NC, USA). The concentrations of Micronutrients content in the digested solutions were determined by inductively coupled plasma atomic emission spectroscopy (ICP-AES, OPTIMA 3300 DV, PerkinElmer, Waltham, MA, USA) (Xia *et al.*, 2019). The all rice genotype used in this study is a newly developed cultivar.

Micronutrients content determination by Optical Emission Spectrometry (OES)

It is based on the principle that atoms of micronutrients content which is normally remain in ground state, under flame condition absorb energy when subjected to radiation is proportional to the specific wavelength. The absorption of radiation is proportional to the concentration of micronutrients. Micronutrients content was estimated in the aliquot of seed extract by using Optical Emission Spectrometry (OES) 200–1100 nm.

Statistical Analysis

Statistical analyses were carried out using SAS 9.4 (USA) software. Analysis of variance was used to determine significant differences between the treatments, with $p < 0.05$ indicating statistical significance.

Results

Micronutrients concentration in various varieties

A minimum of three replications from each of the rice genotypes were analyzed for micronutrients content. The acid method of digestion was followed. The mean of the three replicates was presented in results (Table 1). The total 70 rice genotypes were analyzed for Zn, Fe, Cu, Mn and Se. The significantly increased maximum Zn concentration was recorded in case of 37.73 $mgkg^{-1}$ while the minimum concentration of Zn was recorded 18.28 $mgkg^{-1}$. The maximum Fe concentration was recorded 23.73 $mgkg^{-1}$ while the minimum concentration of Fe was recorded 8.68 $mgkg^{-1}$. The revealed maximum Cu concentration was recorded 4.99 $mgkg^{-1}$ while the minimum concentration of Cu was recorded 1.34 $mgkg^{-1}$. The maximum Mn concentration was recorded 54.16 $mgkg^{-1}$ while the minimum concentration of Mn was recorded 17.93 $mgkg^{-1}$ and the maximum Se concentration was recorded 0.56 $mgkg^{-1}$ while the minimum concentration of Se was recorded 0.03 $mgkg^{-1}$ (Table 1).

Table 1. Average performance of 70 rice genotypes based on Zinc, Iron, Copper, Manganese and Selenium concentration mgkg^{-1}.

S. No.	Genotype	Zn Concentration mgkg^{-1}	Genotype	Fe Concentration mgkg^{-1}	Genotype	Cu Concentration mgkg^{-1}	Genotype	Mn Concentration mgkg^{-1}	Genotype	Se Concentration mgkg^{-1}
1	ZJ-14	37.73^{X} ± 0.8^{Y}	YR-5	23.73^{X} ± 0.4^{Y}	ZJ-11	4.99^{X} ± 0.2^{Y}	ZJ-17	54.16^{X} ± 0.7^{Y}	ZJ-22	0.56^{X} ± 0.2^{Y}
2	ZJ-11	36.46 ± 0.6	YR-27	21.19 ± 0.2	YR-13	4.87 ± 0.1	YR-14	42.56 ± 0.6	ZJ-31	0.51 ± 0.1
3	ZJ-34	34.14 ±1.0	YR-10	21.19 ± 0.9	YR-3	4.77 ± 0.1	YR-19	40.81 ± 0.6	ZJ-3	0.45 ± 0.3
4	YR-28	32.96 ± 0.6	YR-11	20.95 ± 0.1	ZJ-14	4.75 ± 0.1	ZJ-25	40.68 ± 0.3	ZJ-19	0.44 ± 0.2
5	YR-3	31.80 ± 0.3	YR-3	20.34 ± 0.8	ZJ-1	4.71 ± 0.7	ZJ-18	39.96 ± 0.8	ZJ-13	0.42 ± 0.1
6	YR-7	31.54 ± 0.5	YR-7	19.83 ± 0.3	YR-28	4.57 ± 0.4	ZJ-1	39.68 ± 0.6	YR-4	0.41 ± 0.1
7	YR-22	31.40 ± 0.4	YR-1	19.54 ± 0.5	YR-25	4.46 ± 0.3	ZJ-31	37.45 ± 0.2	ZJ-14	0.41 ± 0.1
8	YR-5	31.17 ± 0.7	YR-30	18.99 ± 1.1	ZJ-39	4.46 ± 0.8	YR-13	37.29 ± 1.3	YR-27	0.40 ± 0.1
9	ZJ-25	31.09 ± 0.7	YR-28	18.11 ± 0.8	ZJ-25	4.41 ± 0.1	YR-12	36.74 ± 0.6	YR-10	0.39 ± 0.2
10	YR-23	30.82 ± 0.3	ZJ-39	17.54 ± 1.7	YR-17	4.41 ± 0.3	ZJ-37	34.69 ± 0.4	ZJ-26	0.38 ± 0.2
11	YR-21	30.43 ± 0.7	YR-25	17.54 ± 0.4	ZJ-40	4.41 ± 0.7	ZJ-36	33.35 ± 1.6	YR-18	0.36 ± 0.3
12	ZJ-38	30.43 ± 0.4	ZJ-1	17.49 ± 0.8	YR-16	4.40 ± 0.4	YR-28	32.66 ± 1.2	YR-8	0.40 ± 0.1
13	YR-13	30.38 ± 0.1	YR-20	17.15 ± 0.6	YR-14	4.34 ± 0.9	YR-11	31.10 ± 1.2	ZJ-11	0.37 ± 0.2
14	YR-30	30.12 ± 1.5	YR-14	16.93 ± 0.6	YR-22	4.14 ± 0.1	ZJ-3	30.55 ± 1.6	ZJ-29	0.35 ± 0.4
15	YR-10	29.32 ± 1.5	ZJ-19	16.89 ± 1.0	ZJ-16	4.03 ± 0.2	ZJ-4	30.43 ± 1.3	ZJ-23	0.35 ± 0.1
16	YR-14	28.88 ± 0.5	ZJ-9	16.86 ± 0.1	YR-9	3.98 ± 0.5	ZJ-20	30.40 ± 0.7	ZJ-12	0.34 ± 0.1
17	ZJ-19	28.85 ± 0.1	ZJ-11	16.84 ± 0.1	YR-12	3.95 ± 0.9	YR-9	29.99 ± 0.6	YR-2	0.33 ± 0.2
18	ZJ-33	28.53 ± 0.4	YR-19	16.41 ± 0.7	YR-20	3.94 ± 0.9	ZJ-34	29.89 ± 0.7	YR-14	0.32 ± 0.2
19	ZJ-37	28.42 ± 1.3	ZJ-14	16.23 ± 0.8	ZJ-24	3.93 ± 0.6	YR-21	29.78 ± 0.5	YR-11	0.34 ± 0.1
20	YR-1	28.28 ± 0.4	ZJ-34	16.13 ± 0.9	YR-5	3.87 ± 0.4	ZJ-38	29.78 ± 0.6	YR-7	0.31 ± 0.2
21	ZJ-12	27.90 ± 0.3	YR-24	15.87 ± 0.8	ZJ-21	3.84 ± 0.2	YR-23	29.75 ± 0.5	ZJ-34	0.34 ± 0.2
22	ZJ-17	27.80 ± 0.9	YR-13	15.78 ± 1.1	YR-21	3.83 ± 0.3	YR-18	29.74 ± 2.2	ZJ-24	0.33 ± 0.2
23	YR-19	27.71 ± 0.3	ZJ-30	15.65 ± 0.5	YR-19	3.83 ± 0.5	ZJ-6	29.58 ± 1.2	YR-20	0.31 ± 0.1
24	ZJ-18	27.64 ± 0.9	ZJ-12	15.59 ± 0.8	ZJ-38	3.83 ± 0.4	YR-1	29.32 ± 0.7	ZJ-20	0.33 ± 0.2

25	YR-12	27.23 ± 0.7	ZJ-13	15.44 ± 0.7	ZJ-31	3.81 ± 0.2	ZJ-7	29.12 ± 0.4	ZJ-33	0.30 ± 0.2
26	ZJ-31	27.03 ± 0.2	ZJ-16	15.24 ± 0.6	YR-27	3.79 ± 0.1	ZJ-11	29.05 ± 0.5	ZJ-21	0.32 ± 0.1
27	YR-20	27.02 ± 0.4	YR-29	14.95 ± 0.9	YR-23	3.76 ± 0.7	ZJ-30	29.03 ± 0.6	ZJ-27	0.32 ± 0.3
28	YR-2	26.87 ± 1.1	YR-15	14.92 ± 0.1	YR-7	3.60 ± 0.2	YR-8	28.97 ± 2.4	ZJ-16	0.27 ± 0.2
29	ZJ-16	26.86 ± 0.2	ZJ-7	14.85 ± 0.4	YR-18	3.58 ± 0.1	ZJ-10	28.45 ± 0.6	ZJ-17	0.27 ± 0.1
30	YR-18	26.82 ± 0.4	YR-21	14.68 ± 0.3	ZJ-37	3.55 ± 0.1	ZJ-39	27.95 ± 0.4	ZJ-37	0.26 ± 0.1
31	ZJ-24	26.75 ± 0.6	ZJ-38	14.68 ± 1.2	ZJ-15	3.54 ± 0.2	YR-25	27.95 ± 1.5	ZJ-25	0.26 ± 0.2
32	ZJ-13	26.68 ± 0.7	YR-22	14.64 ± 0.1	ZJ-22	3.54 ± 0.6	ZJ-14	27.75 ± 0.1	ZJ-32	0.25 ± 0.7
33	ZJ-21	26.66 ± 0.7	ZJ-3	14.58 ± 0.4	ZJ-20	3.52 ± 0.3	ZJ-8	27.34 ± 0.5	YR-3	0.25 ± 0.8
34	ZJ-40	26.57 ± 0.6	ZJ-6	14.53 ± 0.4	ZJ-12	3.35 ± 0.4	YR-7	27.28 ± 0.6	YR-21	0.30 ± 0.9
35	YR-17	26.57 ± 0.1	YR-26	14.44 ± 0.5	ZJ-28	3.28 ± 0.7	ZJ-33	27.25 ± 0.6	ZJ-28	0.24 ± 1.1
36	ZJ-22	26.57 ± 0.8	YR-17	14.38 ± 0.3	YR-11	3.19 ± 0.4	ZJ-5	27.07 ± 0.2	ZJ-38	0.24 ± 0.1
37	ZJ-9	26.37 ± 0.1	ZJ-40	14.38 ± 0.5	ZJ-35	3.19 ± 0.2	ZJ-35	26.96 ± 0.8	ZJ-18	0.23 ± 0.1
38	YR-16	26.27 ± 0.1	YR-23	14.21 ± 0.3	YR-10	3.17 ± 0.1	YR-26	26.80 ± 0.8	ZJ-15	0.23 ± 0.2
39	YR-24	25.99 ± 1.2	YR-2	14.20 ± 0.6	ZJ-36	3.17 ± 0.2	ZJ-27	26.69 ± 0.9	ZJ-9	0.22 ± 0.1
40	ZJ-1	25.95 ± 0.9	YR-12	14.12 ± 0.5	ZJ-13	3.16 ± 0.1	ZJ-24	26.63 ± 0.7	YR-6	0.19 ± 0.9
41	ZJ-20	25.53 ± 0.5	ZJ-17	13.99 ± 0.2	ZJ-17	3.16 ± 0.1	ZJ-9	26.33 ± 1.1	YR-26	0.18 ± 0.7
42	ZJ-36	25.45 ± 0.4	ZJ-8	13.98 ± 0.5	YR-2	3.03 ± 0.1	YR-20	26.21 ± 0.5	ZJ-7	0.18 ± 0.6
43	ZJ-2	25.34 ± 0.6	ZJ-20	13.92 ± 0.4	YR-24	3.03 ± 0.2	YR-5	25.61 ± 0.3	YR-15	0.28 ± 0.5
44	YR-11	24.76 ± 0.1	ZJ-31	13.90 ± 0.1	ZJ-23	2.92 ± 0.3	YR-16	25.40 ± 0.3	ZJ-8	0.18 ± 0.1
45	ZJ-39	24.67 ± 0.6	YR-18	13.84 ± 0.2	YR-8	2.85 ± 0.6	YR-24	25.35 ± 0.8	YR-23	0.21 ± 0.2
46	YR-25	24.67 ± 0.8	ZJ-15	13.81 ± 0.6	YR-29	2.76 ± 0.3	ZJ-16	25.10 ± 0.4	ZJ-1	0.20 ± 0.2
47	YR-27	24.27 ± 0.3	YR-16	13.74 ± 0.1	YR-30	2.71 ± 0.6	YR-22	24.98 ± 1.5	YR-16	0.17 ± 0.2
48	ZJ-15	24.23 ± 0.6	YR-6	13.64 ± 0.4	YR-15	2.66 ± 0.1	YR-29	24.53 ± 1.8	YR-9	0.17 ± 0.2
49	YR-6	24.18 ± 0.4	ZJ-24	13.63 ± 0.2	ZJ-32	2.61 ± 0.2	ZJ-2	24.43 ± 0.5	ZJ-35	0.16 ± 0.2
50	YR-26	23.80 ± 0.2	ZJ-22	13.44 ± 0.6	ZJ-34	2.61 ± 0.9	ZJ-29	24.37 ± 0.8	ZJ-4	0.16 ± 0.2
51	ZJ-30	23.56 ± 1.1	YR-8	13.43 ± 0.7	YR-26	2.59 ± 0.1	YR-3	24.25 ± 0.7	YR-29	0.20 ± 0.1

52	YR-15	23.55 ± 0.1	ZJ-21	13.38 ± 0.1	ZJ-8	2.44 ± 0.4	YR-6	23.97 ± 0.7	ZJ-10	0.16 ± 0.2
53	ZJ-32	23.45 ± 0.4	ZJ-10	13.27 ± 0.3	ZJ-5	2.39 ± 0.3	ZJ-26	23.88 ± 0.4	YR-28	0.16 ± 0.1
54	ZJ-3	23.42 ± 0.6	ZJ-18	13.25 ± 0.7	ZJ-29	2.32 ± 0.8	YR-30	23.71 ± 0.7	ZJ-6	0.22 ± 0.2
55	ZJ-27	23.10 ± 0.4	YR-9	13.11 ± 0.3	YR-1	2.29 ± 0.1	ZJ-22	23.60 ± 0.8	ZJ-36	0.15 ± 0.1
56	ZJ-28	22.97 ± 0.4	ZJ-37	13.11 ± 1.2	ZJ-18	2.24 ± 0.4	YR-27	23.61 ± 0.6	YR-22	0.15 ± 0.1
57	YR-9	22.63 ± 0.4	ZJ-2	12.64 ± 0.4	ZJ-6	2.21 ± 0.3	YR-17	23.34 ± 0.9	YR-1	0.14 ± 0.1
58	ZJ-6	22.51 ± 0.8	ZJ-32	12.53 ± 0.3	YR-4	2.09 ± 0.2	ZJ-40	23.33 ± 0.8	YR-12	0.14 ± 0.1
59	ZJ-4	22.00 ± 0.2	YR-4	12.26 ± 0.2	ZJ-4	1.98 ± 0.9	ZJ-21	22.74 ± 1.8	YR-24	0.13 ± 0.2
60	ZJ-10	21.57 ± 0.7	ZJ-33	12.19 ± 1.7	ZJ-7	1.95 ± 0.1	ZsJ-13	22.61 ± 0.9	YR-5	0.13 ± 0.3
61	ZJ-8	21.21 ± 0.8	ZJ-4	12.03 ± 0.2	ZJ-19	1.94 ± 0.5	ZJ-15	22.24 ± 0.9	ZJ-5	0.13 ± 0.2
62	ZJ-35	21.00 ± 1.4	ZJ-36	12.01 ± 0.4	YR-6	1.90 ± 0.2	ZJ-32	22.21 ± 0.5	YR-13	0.12 ± 0.6
63	YR-8	20.59 ± 0.3	ZJ-5	11.77 ± 0.3	ZJ-10	1.83 ± 0.1	YR-2	21.82 ± 0.8	YR-19	0.09 ± 0.2
64	ZJ-7	20.54 ± 0.9	ZJ-35	10.86 ± 0.8	ZJ-33	1.76 ± 0.2	YR-4	21.81 ± 0.6	ZJ-30	0.08 ± 0.7
65	YR-29	20.50 ± 0.5	ZJ-23	9.89 ± 0.5	ZJ-30	1.50 ± 0.2	ZJ-23	21.06 ± 0.7	ZJ-2	0.08 ± 0.2
66	YR-4	20.48 ± 1.8	ZJ-28	9.48 ± 0.1	ZJ-3	1.49 ± 0.1	YR-15	20.51 ± 0.5	YR-17	0.06 ± 0.7
67	ZJ-5	20.37 ± 0.6	ZJ-27	9.29 ± 0.2	ZJ-2	1.42 ± 0.1	ZJ-12	19.76 ± 0.8	ZJ-40	0.06 ± 0.2
68	ZJ-23	20.09 ± 0.3	ZJ-29	9.08 ± 0.9	ZJ-9	1.39 ± 0.2	ZJ-19	19.46 ± 0.5	YR-25	0.05 ± 0.1
69	ZJ-26	18.70 ± 0.8	ZJ-26	8.73 ± 0.7	ZJ-27	1.38 ± 0.2	YR-10	18.57 ± 0.4	ZJ-39	0.05 ± 0.2
70	ZJ-29	18.28 ± 0.2	ZJ-25	8.68 ± 0.1	ZJ-26	1.34 ± 0.9	ZJ-28	17.94 ± 0.6	YR-30	0.04 ± 0.2
Minimum		18.28	Min	8.68	Min	1.34	Min	17.94	Min	0.04
Maximum		37.73	Max	23.73	Max	4.99	Max	54.16	Max	0.56
SED		4.03	SE	3.03	SE	1.00	SE	6.30	SE	0.12
Mean		26.25	Mean	14.80	Mean	3.21	Mean	28.11	Mean	0.25
CV%		1.98	CV%	1.98	CV%	1.98	CV%	1.98	CV%	1.98
LSD at 5%		1.98	LSD at 5%	1.80	LSD at 5%	0.36	LSD at 5%	2.31	LSD at 5%	0.03

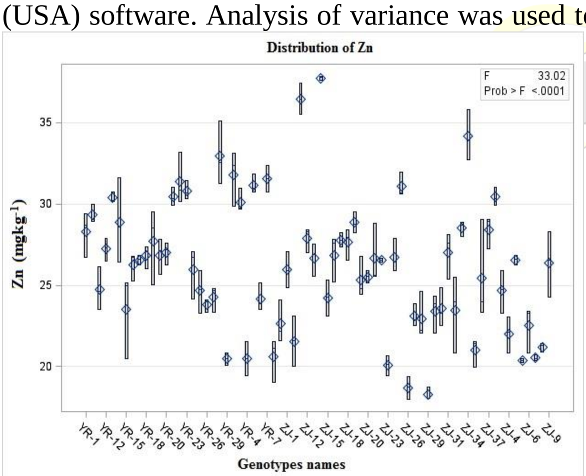
x ± y; where x = mean and y = standard error of mean, SED standard error of difference, CV coefficient of variation, LSD least significant difference. Analysis of variance (ANOVA) was constructed to determine the variations among the concerned genotypes (Table 1), significant at P < 0.05.

Genotypic variations in micronutrients concentrations

1. **Zinc Micronutrient**

A comprehensive range of variation was observed among 70 rice genotypes for Zn concentration. The survey of data revealed that variance due to genotypes was highly significant for the Zn concentration (Fig. 1). This suggested that there were inherent genetic variances among the genotypes. Significant genetic difference for Zn content exhibited by the genotypes showed this character might be effective for further crop improvement. Total 70 rice genotypes were analyzed for Zn concentration. Zinc concentration ranged from 18.28 mgkg^{-1} to 37.73 mgkg^{-1}. Among the 70 rice genotypes i.e. ZJ-14, ZJ-11, ZJ-34, YR-28, YR-3, YR-7, YR-22 and YR-5 had showed the highest Zn content and YR-8, ZJ-7, YR-29, YR-4, ZJ-5, ZJ-23, ZJ-26 and ZJ-29 had showed the lowest Zn content.

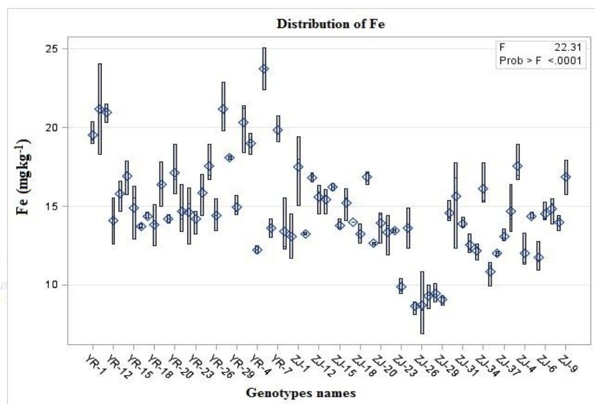
Figure 1. Rice Zn concentration of 70 Genotypes variations of Zn concentrations was constructed to determine the variations among the concerned genotypes. All data are expressed using SAS 9.4 (USA) software. Analysis of variance was used to determine significant differences with $p < 0.05$ indicating statistical significance.



2. **Iron Micronutrient**

Among 70 rice genotypes concentration the wide range of variation was observed for Fe concentration. The review of Fe content data revealed that difference due to genotypes was highly significant for the Fe concentration (Fig. 2). This suggested that there were inherent genetic variances among the genotypes. Significant genetic variation for Fe concentration exhibited by the genotypes indicated this character might be effective for further crop improvement. Total 70 rice genotypes were analyzed for Fe concentration. Fe concentration ranged from 8.68 mgkg^{-1} to 23.73 mgkg^{-1}. Among the 70 rice genotypes i.e. YR-5, YR-27, YR-10, YR-11, YR-3, YR-7, YR-1 and YR-30 had showed the highest Fe content and ZJ-5, ZJ-35, ZJ-23, ZJ-28, ZJ-27, ZJ-29, ZJ-26 and ZJ-25 had showed the lowest Fe content.

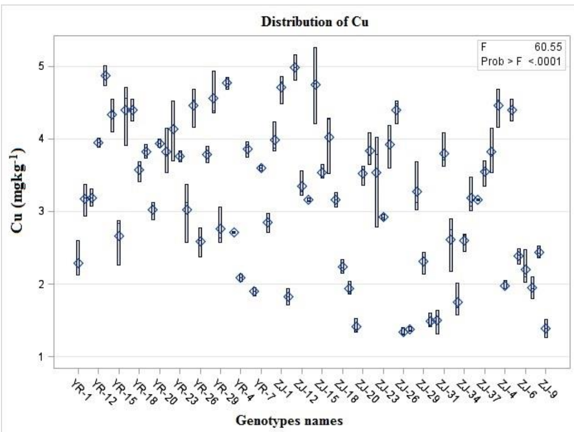
Figure 2. Rice Fe concentration of 70 Genotypes variations of Fe concentrations was constructed to determine the variations among the concerned genotypes. All data are expressed using SAS 9.4 (USA) software. Analysis of variance was used to determine significant differences with $p < 0.05$ indicating statistical significance.



3. **Cupper Micronutrient**

The content varied range of variation was observed among 70 rice genotypes for Cu concentration. The study of data revealed that variance due to genotypes was highly significant for the Cu concentration (Fig. 3). This demonstrate that there were inherent genetic differences among the genotypes. Significant genetic variation for Cu concentration exhibited by the genotypes indicated this character might be effective for further crop improvement. Total 70 rice genotypes were analyzed for Cu concentration. Copper concentration reached from 1.34 mgkg^{-1} to 4.99 mgkg^{-1}. Among the 70 rice genotypes i.e. ZJ-11, YR-13, YR-3, ZJ-14, ZJ-1, YR-28, YR-25 and ZJ-39 had showed the highest Cu content and ZJ-10, ZJ-33, ZJ-30, ZJ-3, ZJ-2, ZJ-9, ZJ-27 and ZJ-26 had showed the lowest Cu content.

Figure 3. Rice Cu concentration of 70 Genotypes variations of Cu concentrations was constructed to determine the variations among the concerned genotypes. All data are expressed using SAS 9.4 (USA) software. Analysis of variance was used to



determine significant differences with $p < 0.05$ indicating statistical significance.

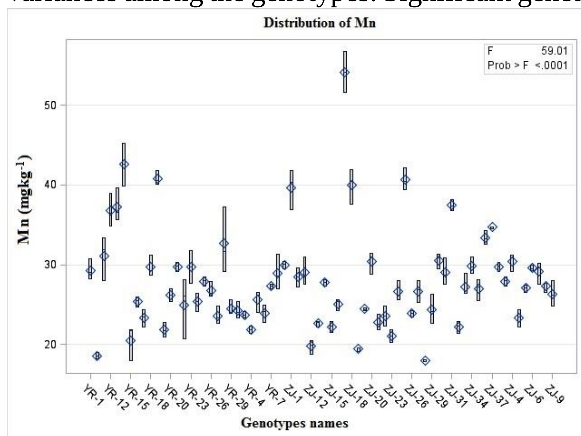
4. **Manganese Micronutrient**

The range of variation was observed between 70 rice genotypes for Mn concentration. The perusal of records revealed that difference due to genotypes was highly significant for the Mn concentration (Fig. 4). This suggested that there were inherent genetic variances among the genotypes. Significant genetic variation for Mn content exhibited by the genotypes indicated this character might be effective for further crop improvement. Total 70 rice genotypes were analyzed for Mn concentration. Manganese concentration observed from 17.94 $mgkg^{-1}$ to 54.16 $mgkg^{-1}$. Among the 70 rice genotypes i.e. ZJ-17, YR-14, YR-19, ZJ-25, ZJ-18, ZJ-1, ZJ-31 and YR-13 had showed the highest Mn content and YR-2, YR-4, ZJ-23, YR-15, ZJ-12, ZJ-19, YR-10 and ZJ-28 had showed the lowest Mn content.

Figure 4. Rice Mn concentration of 70 Genotypes variations of Mn concentrations was constructed to determine the variations among the concerned genotypes. All data are expressed using SAS 9.4 (USA) software. Analysis of variance was used to determine significant differences with $p < 0.05$ indicating statistical significance.

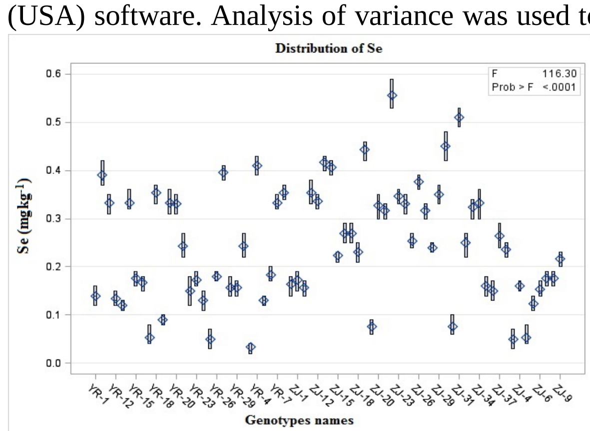
5. **Selenium Micronutrient**

The variation was observed among 70 rice genotypes for Se concentration the perusal of data exposed that variance due to genotypes was highly significant for the Se concentration (Fig. 5). This suggested that there were inherent genetic variances among the genotypes. Significant genetic



variation for Se content exhibited by the genotypes indicated this character might be effective for further crop improvement. Total 70 rice genotypes were analyzed for Se concentration. Selenium content ranged from 0.04 $mgkg^{-1}$ to 0.56 $mgkg^{-1}$. Among the 70 rice genotypes i.e. ZJ-22, ZJ-31, ZJ-3, ZJ-19, ZJ-13, YR-4, ZJ-14 and YR-27 had showed the highest Se content and YR-19, ZJ-30, ZJ-2, YR-17, ZJ-40, YR-25, ZJ-39 and YR-30 had showed the lowest Se content.

Figure 5. Rice Se concentration of 70 Genotypes variations of Se concentrations was constructed to determine the variations among the concerned genotypes. All data are expressed using SAS 9.4 (USA) software. Analysis of variance was used to



determine significant differences with $p < 0.05$ indicating statistical significance.

Discussion

Plant breeding programs in biofortification of staple food crops such as rice and wheat require screening of rice, varieties and commercial rice genotypes lines having micronutrient dense grains to be used as donor parents (Stangoulis, 2010). An increase in concentration of micronutrient in rice grain is a high-priority research area. Exploitation of large genetic variation for micronutrient existing in cereal germplasm is an important approach to minimize the extent of micronutrient deficiencies in developing world. Maximum micronutrients are frequently present in some landraces and /or genetically distant wild varieties (Agarwal *et al.,* 2012).

Hitherto, genetic variability for Zn and Fe contents has been researched in various crops with the aim to identify the donor genotypes that have micronutrient dense grains. Approximately fourfold variation in rice grain Fe content was identified in a research that evaluated 939 genotypes with Fe content spanning between 7.5 and 24.4 mgkg^{-1} and Zn content between 15.9 and 58.4 mgkg^{-1} in brown rice (Graham *et al.,* 1999). Manifold variations in Zn and Fe content in 192 varieties of brown rice were recently reported by Nachimuthu *et al.,* (2014). A screening study among 84 landraces by (Roy, 2014), found that Fe content ranged from 0.25 to 34.8 mgkg^{-1} and Zn content from 0.85 to 195.3 mgkg^{-1}. In a study where 1138 genotypes were screened by (Gregorio, 2002), Fe ranged from 6.3 to 24.4 mgkg^{-1} while Zn from 15.3 to 58.4 mg/kg. In the current study, the concentration of both micronutrients is well within the range as reported by (Gregorio, 2002). This study finds a moderate positive correlation implying the possibility of concurrent selection of the micronutrients. The content of Cu in the rice genotypes ranged from 10.79 to 23.65 mgkg^{-1}, with an average of 16.85 mgkg^{-1}, and the Cu content in the rice was in the range of 3.65 to 8.43 mgkg^{-1}, with an average of 5.68 mgkg^{-1}, which was much lower than that in the rice genotypes (Dengfeng *et al.,* 2016). This difference demonstrated that the element Cu was more likely to accumulate in rice genotypes, consistent with previous research findings (Kang and Xie, 2006). Manganese deficiency has emerged as a serious nutritional issue in global crop production systems (Yang *et al.,* 2007). However, deficiency has become a continuous problem in medium and coarse textured alkaline calcareous soils where rice–wheat rotation is being followed for the last several years (Nayyar *et al.,* 2001). As rice is staple food for billions, increasing grain Mn contents the grain biofortification is a pragmatic and cost-effective affective method for Mn nutrition in human. Agronomic biofortification is a way of increasing the grain nutrient contents with the targeted nutrient through different approaches such as soil application, foliar application and seed treatments. These agronomic biofortification approaches increase the genetic or breeding efforts (Ullah *et al.,* 2016). Se rice grain (0.04–0.56 mgkg^{-1} Se) concentration can be produced in 70 rice genotypes. Se enriched food is an efficient resource for human dietary Se intake (Carey *et al.,* 2012; Williams *et al.,* 2009). However, under high Se treatment, Se concentration in rice was 0.04–0.56 mgkg^{-1} (Table 1), respectively, which was higher than the maximum standard of Se concentration (0.3 mg kg^{-1}) in cereal (Sun *et al.,* 2010). The tolerable upper Se intake level recommended by the WHO for adults is 400 μg day^{-1} (Huang *et al.,* 2013). The rice which were grown in paddy soil with total Se up to 1.5 mgkg^{-1}, were not suitable for daily consumption of humans, unless diluted with Se deficient rice to meet the standard (≤ 0.3 mgkg^{-1}) (Sun *et al.,*2010). Lots of studies showed that Se enriched rice was achieved by spraying Se fertilizer (Carey *et al.,* 2012). However, Se concentration in polished rice and brown rice considerably exceeded 0.3 mgkg^{-1}. In order to achieve Se enriched rice within the standard of Se concentration 0.3 mgkg^{-1} by spraying Se, more studies should be carried to investigate optimal spray concentration of Se. Nonetheless, our results suggested that micronutrients enriched rice could be produced in natural conditions in the study area, which will be more feasible and environmentally friendly as exogenous micronutrients pollution is avoided. Understanding the percentage distributions of micronutrients in the different fractions of the grain is important for the efficient use of rice. However, little study has been conducted using these techniques so far, especially in China, to value the bioavailability of the increased micronutrients concentration in edible parts of crops that is what we want to study in the near future.

Conclusion

Identification of rice genotypes for Micronutrients content is the initial step of biofortification. The highest Zn content 37.73 mg kg^{-1} was recorded in ZJ-14 genotype and lowest 18.28 mg kg^{-1} in ZJ-29. The highest Fe 23.73 mg kg^{-1} content was found in YR-5 genotype and lowest 8.68 mg kg^{-1} in ZJ-25. However, the highest Cu content 4.99 mg kg^{-1} was found in ZJ-11 genotype and lowest 1.34 mg kg^{-1} in ZJ-26. In case of the highest Mn content 54.16 mg kg^{-1} was recorded in ZJ-17 mg kg^{-1} genotype and lowest 17.93 mg kg^{-1} in ZJ-28. The ZJ-22 genotype was revealed the highest Se content 0.56 mg kg^{-1} and YR-30 was found in lowest contents 0.03 mg kg^{-1} of Se. Hence, from the results it shows the variation among the selected commercial rice genotypes for micronutrients status in grains and indicated the different behavior of the genotypes for the uptake of micronutrients. Furthermore, among the screened materials, the genotypes having higher and lower of micronutrients content can be used as a breeding material for biofortification process in future.

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