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Detection and Analysis of Mineral Elements in Giant Embryo Rice

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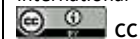
Abstract

Mineral elements are essential micronutrients necessary to constitute human tissue and maintain normal physiological functions. Micronutrient deficiencies are an important issue currently faced in the world. The best strategy to solve this problem is to breed special functional types of rice varieties rich in trace elements (special rice for short). A special type of rice that can produce rich nutrients. In order to explore the nutritional value of giant embryo rice, the contents of 17 mineral elements ((K, Ca, Fe, Se, Zn, Cu, Na, Mn, Mg, Ni, Cr, Co, Pb, As, Cd, Ag, Al)) in 5 kinds of giant embryo rice (white giant embryo, giant japonica-GB-12, giant japonica-GB-11 and giant japonica-GB-5, giant japonica-GB-1) were detected by inductively coupled plasma mass spectrometry. After comparison with ordinary rice, it was found that the

content of the same mineral element in different samples has a large variation, suggesting that different rice varieties have different absorption, transformation and storage efficiency of the same element; different giant embryo brown rice in some mineral elements (such as Cr, Ni, Cu and Zn) are extremely low in content and almost undetectable. Meanwhile, the brown rice of giant embryo rice has a more powerful enrichment effect on some mineral elements, which means that embryo rice has a unique advantage in cultivating new rice varieties with special functions rich in minerals.

Keywords: Giant embryo rice; Minerals; Special rice; ICP-MS.

1. Introduction

The cultivation history of rice (*Oryza sativa* L.) can be traced back to as early as 12000 ~ 16000 years ago [1]. It can be said that rice has accompanied the development of human civilization. As one of the oldest and most important food crops, the sown area of rice accounts for about 23% of the world's total food crops, but the yield of rice reaches 29% of the total output of food crops. More than half of the world's population and two-thirds of China's population eat rice as the staple food, and rice provides more than 25% of the energy needs of humans [2, 3]. With the rapid development of the global economy and the continuous improvement of people's living standards, People's demand for food has changed from past satiety to now safe and pollution-free, and the pursuit of food nutrition and health [4], however, in human diet, the lack of minerals is widespread [5]. It is estimated that about 2 billion or even half of the world's population suffers from bioavailable micronutrient deficiency, especially in developing countries. This problem is more prominent [6-9]. In the vast majority of developing countries, for those who take rice as the staple food, rice not only provides them with a large part of their daily caloric intake, but also an important source of vitamins and minerals.

Rice as a staple food may lack key nutrients, so people can get enough calories by eating rice as a staple food, but not enough nutrients and minerals. This situation is called "hidden hunger". Specifically, recessive hunger means that the quality of food that people eat does not meet the nutritional needs of human growth and development. Mahender et al. Found that the main cause of hidden hunger in developing countries is that most of the rice that people eat is polished rice [10]. Polished rice is the product of polished brown rice. The nutrients and minerals in rice are mainly concentrated in the embryo and aleurone layer of brown rice. For example, iron is mainly found in the scutellum, aleurone layer and embryo of rice [11]. In order to cope with bioavailable micronutrient deficiency, it is necessary to develop more nutritious rice varieties, and giant embryo rice is a kind of rice that can produce high nutrients in special rice. Giant embryo rice has nearly 40 years of research history and is a fairly mature special type of rice variety. Compared with ordinary rice, the embryo body of giant embryo rice increases about 2 to 3 times, and the content of protein, minerals and vitamins in them is high. More importantly, the embryo and seed coat of giant embryo rice are very rich Mineral elements.

As an essential element of human body, mineral element is not only an important raw material to constitute human tissue, but also necessary to maintain the normal physiological function of human body, and the amount of mineral element in human body will also affect human intelligence and mood, which is the material basis of human mental health, so it is called "life element". Once trace mineral elements are deficient or disproportionate in the human body, they will cause various physiological changes and various pathologies or certain diseases [12]. With the development of modern science and technology, people can supplement minerals by pharmaceutical supplements, but compared with supplementing minerals by drugs, supplementing minerals by diet has unparalleled advantages in terms of safety and absorption rate. Therefore, breeding mineral-rich special rice is an important way to solve the problem of mineral deficiency in human diet.

Research on the minerals in special rice has been carried out for decades, and a series of important progress has been made. At present, there are more than 100 kinds of special rice varieties rich in minerals cultivated, and there are more than ten kinds of rice rich in minerals on the market. However, compared with the huge demand of the masses of people, there is still a big gap in the achievement of the cultivation of rice rich in minerals. Therefore, in this study, 17 mineral elements (K, Ca, Fe, Se, Zn, Cu, Na, Mn, Mg, Ni, Cr, Co, Pb, As, Cd, Ag and Al) were detected and analyzed by inductively coupled plasma mass spectrometry (ICP-MS) in five giant embryonic rice species in the experimental field, with a view to providing reference and reference for the cultivation of new rice varieties rich in minerals.

2. Materials and Methods
2.1. Planting of Test Materials and Related Reagents

Five kinds of giant embryo rice (white giant embryo, giant japonica-GB-12, giant japonica-GB-11 and giant japonica-GB -5, giant japonica-GB-1) were selected as the test materials in the experimental field of Xinyang Normal University, and Zhonghua 11, a common japonica rice, was used as the control. Each rice variety is planted in 2 rows with 12 plants in each row, and the spacing between plants is 16.5 cm × 26.4 cm. From the sowing to the final seed maturity of the test materials, the ordinary field routine cultivation and management are carried out, the shallow water layer is maintained in the field during the whole growth period, and diseases, pests and weeds are strictly controlled until harvesting at maturity.

Ultrapure water, nitric acid: φ (HNO$_3$) = 65%, hydrogen peroxide: φ (H$_2$O$_2$) = 30%, argon (Ar): high purity argon (> 99.99%), element stock solution (1000 mg · L^{-1}) (K, Ca, Fe, Se, Zn, Cu, Na, Mn, Mg, Ni, Cr, Co, Pb, As, Cd, Ag, Al), internal standard solution (1000ug · L^{-1}) (Ge). Take 3 ml of concentrated nitric acid, slowly add it to 27 ml of Ultrapure water, mix well and make a nitric acid solution.

2.2. Main Equipment

Thresher (5TS-150A type), cyclone mill, analytical balance (sensitivity 0.1 mg), inductively coupled plasma mass spectrometer (ICP-MS), microwave digestion instrument (with PTFE digestion tank, with control Moderate pressure regulation function).

2.3. Test Methods
2.3.1. Sample Pre-Processing

After the five giant embryo varieties and their control materials used for testing were ripe and harvested, they were naturally dried and processed by a single plant thresher. After the rice seeds were placed at room temperature for 3 months, the rice husks of the rice seeds were separately removed by a ridge mill, and then the brown rice material was crushed by a cyclone mill, and then crushed and passed through a 100-mesh sieve to make rice flour for use.

2.3.2 .Dissolution Treatment

Weigh 0.2 g of the test rice flour, place it in the PTFE inner tank, add 5 ml of the prepared nitric acid solution and 2 ml of H_2O_2 solution, cover the inner cover, soak for 30 min, install a protective cover, put the digestion tank into the microwave digestion instrument, set up a micro-digestion program (see Table 1 for the microwave digestion conditions of the sample), and start the digestion treatment.

Table-1. Operation conditions of microwave digestion

step	1	2	3
Power/kW	800/1600	800/1600	800/1600
Temperature/℃	120	160	190
Temperature rise time/min	6	4	4
Hold time/min	10	10	20

2.3.3. Post-Digestion Processing

After the digestion is completed, the inner tank is taken out, and the digestion solution becomes a pale-yellow transparent liquid without residue. The digestion solution in the inner tank was washed with ultra-pure water a few times and transferred to a 100 ml volumetric flask, constant volume, and mixed. Perform another gradient dilution. After dilution, use a 15 ml large centrifuge tube to take 10 ml of the sample solution and blank reagent, put it into an inductively coupled plasma mass spectrometer (ICP-MS), and set the operating parameters of the inductively coupled plasma mass spectrometer (see Table 2 for working parameters), The sample is automatically loaded for analysis.

Table-2. operation conditions of inductively coupled plasma mass spectrometer

Instrument parameters	Numerical value
RF power	1500 W
Plasma gas flow	15.00 L · min^{-1}
Carrier gas flow	1.18 L · min^{-1}
Auxiliary gas flow	0.10 L · min^{-1}
Ammonia flow	4.5 mL · min^{-1}
Spray chamber temperature	2 ℃
Sample lifting rate	0.3 r · s^{-1}
Sample lift	0.4 mL · min^{-1}
Nebulizer / spray chamber	High-salt atomizer / concentric atomizer
Sampling cone / intercepting cone	Nickel cone
Sampling depth	8 mm
Acquisition mode	spectrum
Detection method	automatic
Determination points per peak	3
repeat times	3

3. Results

In the six rice samples tested, a total of 17 mineral elements were detected, of which only 4 elements (Na, K, Mg, Ca) were detected in all samples (Table 3). A total of 12 mineral elements of Al, Mn, Fe, Co, As, Se, Cd, Pd, Na, K, Mg and Ca were also detected in white giant embryo rice; 8 mineral elements of As, Ag, Cd, Pd, Na, K, Mg and Ca were detected in giant japonica-GB-12; only 4 minerals of Na, K, Mg and Ca were detected in giant japonica-GB-11 samples; A total of 12 mineral elements, including Al, Mn, Fe, Co, As, Ag, Cd, Pd, Na, K, Mg and Ca, were detected in giant japonica-GB-5, and 12 mineral elements, including Al, Mn, Co, As, Se, Ag, Cd, Pd, Na, K, Mg and Ca, were detected in giant japonica-GB-1.In the control samples, ten mineral elements including Mn, Fe, Co, As, Cd, Pd, Na, K, Mg and Ca were detected (Table 3). Interestingly, the content of the same mineral element varies greatly in different samples, suggesting that different rice varieties have different absorption, transformation

and storage efficiency for the same element. This characteristic will provide important information for the cultivation of new varieties of special rice rich in a particular mineral nutrient.

Among the 17 mineral elements detected in the six rice samples tested, Cr, Ni, Cu and Zn were not detected (Table 3), which indicated that the contents of Cr, Ni, Cu and Zn were very low or not at all in rice. In the white giant embryo samples, 5 mineral elements, Ag, Cr, Ni, Cu, Zn, were not detected; 9 kinds of mineral elements Al, Mn, Fe, Se, Co, Cr, Ni, Cu, Zn were not detected in giant japonica-GB-12; giant japonica-GB-11 did not detect Al, Mn, Fe, Co, As, Se, Ag, Cd, Pb, Cr, Ni, Cu, Zn 13 kinds of mineral elements; 5 mineral elements of Se, Cr, Ni, Cu, Zn were not detected in giant japonica-GB-5; 5 mineral elements of Fe, Cr, Ni, Cu, Zn were not detected in giant japonica-GB-1; control sample A total of 8 mineral elements including Al, Co, Se, Ag, Cr, Ni, Cu, and Zn were not detected. It can be inferred from this that the absorption, transformation and storage efficiency of Ag by white giant embryos is very low; the absorption, transformation and storage efficiency of Al, Mn, Fe, Se and Co by giant japonica-GB-12 is very low; The absorption, transformation and storage efficiency of Al, Mn, Fe, Co, As, Se, Ag, Cd and Pb by Giant Japonica-GB-11 is extremely low; the absorption, transformation and storage efficiency of Se by Giant Japonica-GB-5 is extremely low; the absorption, transformation and storage efficiency of Fe by Giant Japonica-GB-1 is very low.

Compared with the control rice, the contents of Na, K, Mg, Ca, Mn, and Fe in the five kinds of giant embryo brown rice increased to varying degrees, and their relative changes were relatively large (Table 3). Compared with the control, the contents of Na, K, Mg, Ca, Mn and Fe in white giant embryo increased by 2694.0%, 303.3%, 1306.1%, 1327.8%, 444.0% and 7.2% respectively; The contents of Na, K, Mg and Ca of giant japonica-GB-12 increased by 53.4%, 50.4%, 253.8% and 82.3% respectively compared with the control; the contents of Na, K, Mg and Ca of giant japonica-GB-11 Compared with the control, the contents increased by 12.4%, 36.0%, 244.5%, and 101.7% respectively; the content of Na, K, Mg, Ca, Mn, and Fe in giant japonica-GB-5 increased by 774.9%, 67.1%, 374.0%, 238.0%, 179.3%, 36.7%; the content of Na, K, Mg, Ca, Mn in giant japonica-GB-1 increased by 478.2%, -16.1%, 102.3%, and 1.6%, 28.4%. Therefore, the contents of Na, K, Mg, Ca, Mn, and Fe in brown rice of giant embryo rice are higher than the control, which has important reference value for the nutritional quality of giant embryo rice.

Table-3. Contents of mineral elements in six kinds of rice

Element	white giant embryo	giant japonica-GB-12	giant japonica-GB-11	giant japonica-GB-5	giant japonica-GB-1	Contrast
Na	554.495	30.490	22.310	173.636	114.757	19.846
Mg	73.993	18.616	18.129	24.943	10.646	5.262
Al	1.584	-	-	8.746	1.997	-
K	85.105	31.738	28.692	35.255	17.712	21.101
Ca	31.897	4.072	4.505	7.550	2.270	2.234
Cr	-	-	-	-	-	-
Mn	1.262	-	-	0.648	0.298	0.232
Fe	0.520	-	-	0.663	-	0.485
Co	0.001	-	-	0.001	0.053	-
Ni	-	-	-	-	-	-
Cu	-	-	-	-	-	-
Zn	-	-	-	-	-	-
As	2.198	0.327	-	5.193	0.125	0.607
Se	0.026	-	-	-	0.052	-
Ag	-	0.001	-	0.004	0.007	-
Cd	0.221	0.002	-	0.068	0.015	0.004
Pb	4.969	0.021	-	2.021	0.016	0.028

Note: "-" means not detected, the unit is mg · kg^{-1}.

4. Discussion

4.1. Selection of Test Method

Inductively coupled plasma mass spectrometry (ICP-MS), as an element-specific detector, is characterized by high sensitivity, low detection limit, wide linear range, strong anti-interference ability, simultaneous analysis of multiple elements and multiple isotopes. Compared with other detection methods, ICP-MS has many advantages [13]. The ability to accurately analyze trace elements in complex matrices is one of the most powerful means for trace element analysis. Nowadays, inductively coupled plasma mass spectrometry is still the core of method research in the field of detection in the detection of heavy metals in food. Moreover, China 's national food safety standard and the Ministry of Agriculture standard have gradually introduced inductively coupled plasma mass spectrometry as an arbitration method [14]. Therefore, the author uses the People's Republic of China grain industry standard LST6136-2019 as a guide to analyze the mineral elements in rice by inductively coupled plasma mass spectrometry. From the test results, this method has a better detection effect.

4.2. Optimization of Digestion System

Zhao, et al. [15], selected 5 ml HNO$_3$, 5 ml HNO$_3$ + 2 ml H$_2$O$_2$, 3 ml HNO$_3$ + 4 ml of H$_2$O$_2$ to determine standard samples (GBW10043). It was found that when the digestion system of 5 ml HNO$_3$ and 3 ml HNO$_3$ and 4 ml H$_2$O$_2$ was used, the determination results of five heavy metal elements were not completely within the standard range, and the recovery rate was 82.3%-95.8%. However, when using 5 ml HNO3 + 2 ml H2O2 digestion system, the determination results of the five heavy metal elements are all within the standard range, and the recovery rate is 99.8% -103.3% [15]. Therefore, we decided to use 5 ml HNO3 + 2 ml H2O2 as the best digestion system for digestion samples.

Studies have shown that the size of microwave power only affects the speed of temperature and pressure rise, so the power selected can only ensure that the pressure in the digestion tank reaches the required pressure within a set time [16]. In addition, when heated for a long time under low temperature conditions, the organic matter is still difficult to be digested completely, and the digestion solution is yellow turbid liquid; when digested at high temperature and high pressure, the reaction is too drastic and acid gas leaks easily, resulting in sample loss. Therefore, first put the sample into the digestion tank for 30 min at room temperature, then put it into the digestion instrument to digest the rice using the digestion working parameters of Table 1, which can achieve a better effect.

4.3. Mineral Elements and Human Health

Na, K, Mg, Ca, Mn and Fe are all beneficial and indispensable elements for human health, among which Na is one of the important elements to maintain the osmotic pressure balance of human intracellular and extracellular fluids. Na has the function of assisting the normal operation of nerve, heart, muscle and various physiological functions in the body, and is also an important component of bile, sweat and tears. If low sodium. It can lead to edema, decrease of blood pressure, coma, etc. Severe patients can endanger life. K is also one of the important elements to maintain the osmotic pressure balance of human intracellular and extracellular fluids. It can regulate the osmotic pressure inside and outside cells, maintain water, regulate acid-base balance. When blood potassium is lower than 3.5 mmol · L^{-1}, symptoms such as fatigue and depression will occur in mild cases, while circulatory failure, quadriplegia and coma will occur in severe cases [17]. Mg can activate more than 300 enzymes in the human body and participate in the life activities of the body. It has the functions of participating in the metabolism and synthesis of energy and macromolecular substances, active transport of substances, transmission and expression of genetic information [18]. When Mg is deficient in the body, emotions tend to be tense, and the normal function of nerve cells is impaired, resulting in migraine. Ca is the most abundant mineral element in human body, accounting for about 1.5%~2% of human body mass, 99% of which is found in bone and teeth, constitutes a human scaffold, and serves as a reservoir for calcium in the body; the rest are distributed in blood, extracellular fluid and soft tissue cells, collectively known as miscible calcium pool, which keeps a dynamic balance with bone calcium. Ca deficiency can easily lead to rickets, leading to osteoporosis and inducing fractures; low calcium can stimulate the production of "hypertensive factors", resulting in high blood pressure Ca deficiency, and it can also cause idiopathic edema, which makes it difficult for people to concentrate and cause memory decline [19]. Mn is one of the essential elements of human body and the activity center of various acids [20]. Mn deficiency mainly manifests as weight loss, dermatitis, nausea, vomiting, hair discoloration and dysplasia, and decreased cholesterol value [21]. As a carrier and a component of enzymes, Fe participates in the composition of hemoglobin, myoglobin and other enzymes [22]. At the same time, Fe is also a cofactor involved in energy metabolism and thermoregulation. Fe deficiency can lead to anemia, irritability, fatigue, immunocompromise, impaired mental activity and intellectual development.

As, Pb and Cd are all heavy metal elements, which are extremely harmful to human body. The acute poisoning symptoms of As are general discomfort, fatigue, headache and dizziness, followed by nausea and vomiting, severe abdominal pain and diarrhea hallucination. The chronic poisoning of As is usually accompanied by abnormal skin pigmentation, slight neuritis and thickening of skin keratosis, which can further develop into skin cancer [23]. Pb has a toxic effect on every system of the body, which can cause disorder of many organ systems, and can cause dizziness, headache, weakness, joint pain, anemia, hypomnesia, neurasthenia and other symptoms. It is harmful to kidney, gastrointestinal tract, cardiovascular system, immune system and endocrine system [24]. The toxic effects of Cd are mainly concentrated in lung and kidney damage. Cadmium ingested through diet mainly causes kidney damage, rickets and fractures [25]. Existing studies have confirmed that Cd has kidney, bone, lung, reproductive and developmental toxicity and other damaging effects. The kidney is the main target organ for long-term cadmium exposure. The International Agency for Research on Cancer (IARC) lists cadmium as a class I carcinogen. According to the GB/T 762-2017 "National Standard for Food Safety-Limits of Contaminants in Foods" issued by China in March 2017, the As element limit in brown rice is 0.2 mg · kg^{-1}, and the Cd element limit is 0.2 mg · kg^{-1}, Pb element limit is 0.5 mg · kg^{-1}. The excess rate of As, Cd and Pb elements in white giant embryos were 999.0%, 10.5% and 893.8%, respectively. The As, Cd and Pb elements in giant japonica-GB-12 did not exceed the standard. The As and Pb elements in giant japonica-GB-5 exceeded the standard by 2496.5% and 304.2%, respectively. The Cd element did not exceed the standard. The over standard rate of As element in giant japonica-GB-1 was 203.5%, and Cd and Pb elements were not over standard. Some rice heavy metal components exceed the standard seriously, especially the As element, which exceeds the national safety limit by 9 to 10 times. For As, Cd, Pb and other harmful heavy metal elements, the accumulation of rice grains can also be alleviated by transgenic and mutant technology. Ishikawa et al. Used carbon ion beam irradiation to obtain three mutant lines, which reduced Cd in grains (<0.05 mg · kg^{-1}) compared to wild type (1.73 mg · kg^{-1}); in all three lines, the mutation was identified as OsNRAMP5. Cd was almost undetectable in the grains of the mutants, and did not show adverse agricultural or economic characteristics

[26]. Also proposed a method to simultaneously reduce arsenic and cadmium in food: Rice plants mutated with OsNRAMP5 gene can reduce As content by 27% and Cd content is almost zero under water management system with alternating wet and dry [27].

4.4. Creation of Special Function Rice

According to the provisions of "Selenium-enriched rice" (GB/T 22499-2008), the selenium content of rice processed with selenium-enriched rice should be between 0.04 mg · kg^{-1} and 0.30 mg · kg^{-1}. Se has the effect of preventing peroxide from denaturation and aging of nucleic acid proteins in cells. When foreign matter invades, it can give full play to the defense function of neutrophils and macrophages, improve immunity and have good preventive and therapeutic effects on cancer [28]. The content of Se element in giant japonica-GB-1 meets the requirements. At the same time, the content of heavy metal elements is far lower than the national safety standards. Therefore, giant japonica-GB-1 is an ideal special rice material for selenium-enriched rice.

Biofortification is the main method to increase the mineral content in rice. Overexpression of the metal chelate gene and metal transporter gene in rice through transgenic technology can greatly increase the mineral content in rice. For example, Lee et al. Found that rice lines overexpressing iron-regulated transporter-like protein 1 (OsIRT1) accumulated more Fe and Zn in seeds [29]. Ishimaru et al. Significantly increased the content of Fe and Mn in rice by overexpressing the metal-nicotianamine transporter (OsYSL2) [30]. In addition to transgenic technology, the application of zinc fertilizer to rice can not only promote rice growth, but also increase the zinc content in rice. At the same time, the problem of heavy metal pollution in giant embryo rice is also very prominent. If it is used as a raw material for the cultivation of special rice with high mineral content, it is necessary to further improve its characteristics while controlling its growth environment.

5. Conclusion

In this paper, based on the People's Republic of China grain industry standard (LST6136-2019), 17 mineral elements in 5 kinds of giant embryo rice (white giant embryo, giant japonica-GB-12, giant japonica-GB-11 and giant japonica-GB-5, giant japonica-GB-1) were detected and analyzed by inductively coupled plasma mass spectrometry. The results showed that the content of the same mineral element in different samples varied greatly, and different rice varieties had different absorption, transformation and storage efficiency for the same element. Compared with ordinary rice, some mineral elements in brown rice of giant embryo rice have more powerful enrichment effect, but some mineral elements (such as Cr, Ni, Cu and Zn) are very low in brown rice of giant embryo rice, which means that giant embryo rice has unique advantages in cultivating new rice varieties with special functions rich in minerals.

Acknowledgments

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