



Research article

Selenium in rice: Impact on protein content and distribution for enhanced food and feed security in agroclimatic challenges

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ABSTRACT

Countries face exasperating and inclement climate worldwide. Food and feed security could be their paramount life objective. The study aimed to investigate the impact of selenium on the protein content and distribution in different parts of rice. For this purpose, advanced selenium biofortified breeding material developed after generations of breeding efforts was investigated at the field area, rice research institute, Chengdu, China during cropping season 2021–22. The accumulation and distribution of selenium and protein contents were observed in various fractions of selenium-enriched rice (Z3057B) and positive control (727). The correlation studies for selenium and protein quantification leads to the optimization of the breeding material and relevance in virtue. The rice fractions indicated rice embryo retains highest selenium contents, which gradually decreases in succession (other rice parts). The difference in protein content between the embryo and endosperm of Se-enriched rice is significant, while that between embryo and aleurone layer is not obvious. The selenium protein was found with molecular weight of 13.6–122.6 kDa. The protein of each molecular weight is found to bind with selenium, but the binding strength of selenium is negatively correlated with the molecular weight of protein. The 67.5% of the total selenium sticks with protein having molecular weight less than 38.8 kDa. In summary, protein with low molecular weight (13.4 kDa) binds maximum selenium and accounts for highest total protein content (40.76%).

1. Introduction

Climate change and food security are emerging issues around the Globe not just for human being but also for the animals. Lack of climate resilient and nutritious germplasm is the biggest concern for the farmer's community. Selenium (Se) is an important trace element, which enhances anti-oxidation activity, improves immunity, health, and delays ageing [1–4] while other health-related

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Abbreviations

Se Selenium
SDS-PAGE sodium dodecyl sulfate polyacrylamide gel electrophoresis

benefits of this element are still under research. The dual effects of selenium depend on the dose, morphology and biological effectiveness of animals and plants [4–6]. Many studies have reported exogenous selenium application could improve selenium-enrichment in products, but their effective absorption and utilization is far less than the organic selenium-enriched materials developed by years of breeding efforts [7,8]. Some studies indicated the hyper-toxicity of selenium in plant parts with no obvious symptoms of necrosis. The selenium concentration must be as per national standards i.e., in range of 0.04–0.30 mg/kg (GB/T 22499-2008), safe for bio-fortification of field crops. The excess of this metal could lead to severe health associated risks.

In rice, different layers of seed (brown rice, polished rice, episperm, aleurone layer, embryo and endosperm) behaved differently for selenium accumulation and transformation [9]. Brown rice is a reservoir of nutrients such as starch, protein, minerals, functional components, dietary fiber, phytic acid, oryzanol and vitamins [10]. It is an important meal having better nutritional value and higher hygienical functions for animals and humans, and various products are made from it [11]. The episperm layer of brown rice could accumulate more phytate, cellulose, and crude residues which left poor taste during chewing and is unpopular. On the other hand, polished rice is a main edible form of rice, delicate in taste and having a variety of nutrients including protein, fat, vitamins and mineral elements mainly in the embryo and episperm [12]. Milling operations significantly reduce the nutrients in polished rice. Many studies have shown the relationship between rice milling degree and nutrient loss. As the protein content decreases, the maximum viscosity and breakdown value (measured by RVA) increase under fine milling of diverse degrees, reducing the setback value. As the polished degree increases in milling, the protein content decreases somewhat. The rice hardness gradually decreases, and the viscosity gradually increases [13]. Therefore, nutrients are in uneven distribution in the various parts of rice grains after continuous milling for brown rice.

The selenium bio-fortification by exogenous means (foliar spraying and fertilization methods) could enhance selenium contents in rice grain as indicated by some field experiments [14–16]. Although it is an effective way to increase Se content (nonetheless, associated with high costs). The impact of long-term sprays causes environmental pollution and is hazardous. Furthermore, its influence on protein architecture and binding strength is not fully explored. Former studies elaborated that selenium biofortified rice developed by breeding have high value by-products. Its extracts have high antioxidant and free radical scavenging ability in vitro and could improve selenium retention, serum oxidative status and growth performance in vivo [17,18]. Therefore, to choose environmentally friendly Se-enriched biofortified rice (safer for the human body) should be one's choice.

The humans and animals feed and food are the prime concern of time, and the existing germplasm needs to be upgraded for quality traits. The study aims to develop an agronomic itinerary for breeding lines of rice that are enriched with selenium, which has been shown to have antioxidant and free radical scavenging abilities, as well as improve selenium retention, serum oxidative status, and growth performance in vivo [3,4]. The proposed objective of the current study includes 1) investigating the binding strength of selenium with different protein structures of varying molecular weights. 2) To figure out the impact of selenium biofortification on

Table 1

The breeding efforts of rice material (Z3057A, Z3057B) responsive for Se.

Year	Location	Breeding rules
Winter 2006	Lingshui, Hainan, P.R. China	(G46B × ruby) F1
Spring 2007	Wenjiang, Sichuan P.R. China	F2, 15 single plants with red rice grain were selected
Winter 2007	Lingshui, Hainan P.R. China	G46A × F3, single plants with good agronomic characteristics and high quality rice were selected to have testcross with G46A to select and preserve plant with required traits
Spring 2008	Wenjiang, Sichuan P.R. China	F1 × F4, single plants with good preservation, excellent rice quality and agronomic characteristics were selected to have backcross with completely sterile high quality single plants
Winter 2008	Lingshui, Hainan P.R. China	BC1 × F5, Quality plants were selected for backcross
Spring 2009	Wenjiang, Sichuan P.R. China	BC2 × F6, Single plants with trim heading, good flowering habit, nice appearance, and high quality were selected for backcross
Winter 2009	Lingshui, Hainan P.R. China	BC3 × F7, One high quality strain was selected for backcross to get maintainer lines
Spring 2010	Wenjiang, Sichuan P.R. China	BC4 × F8, The strain numbered Z3057 was selected for backcross to develop maintainer lines. The demonstrative planting, trails, and combination ability determinations of these sterile line were conducted
Winter 2010	Lingshui, Hainan P.R. China	BC5 × F9, One quality strain was selected for backcross to develop maintainer and sterile lines while breeding efforts were made on small area for seed production purposes
Spring 2011	Wenjiang, Sichuan P.R. China	BC6 × F10, The breeding of sterile lines for plant's population expansion and seed production on small area
Winter 2011	Lingshui, Hainan P.R. China	BC7 × F11, The breeding of sterile and maintainer lines for plant's population expansion and seed production on small area
Spring 2012	Wenjiang, Sichuan P.R. China	Z3057A, Z3057B

protein content, physiological, biochemical, and quality characteristics along with binding strength in rice breeding research material of variable features (selenium enriched and non-enriched rice). The study will provide a scientific basis for the safe use of selenium in agricultural production.

2. Materials and methods

2.1. Plant materials

The study was conducted at Sichuan Agricultural University, Chengdu P.R. China during the rice growing season of 2021–2022. Rice research material responsive for selenium developed after years of breeding work (Table 1) i.e., Genotype with high Se uptake rice (Z3057B) and Genotype with low Se uptake rice (positive control) rice (Chenghui727), collected from Demonstration base for international science and technology cooperation of Sichuan province, Rice Research Institute. The selenium contents in both rice cultivars were analyzed by dual-channel atomic fluorescence analyzer (RGF-6800, Bo Hui Co., Ltd., Beijing, P. R. China) following standards (GB-5009.93-2017). The selenium concentration in selenium-rich rice meets the national standard of selenium-rich paddy, that is, 0.04–0.30 mg/kg (GB/T 22499-2008). The selenium concentration in brown rice of Z3057B was (0.046 mg/kg) and in Chenghui727 was 0.022 mg/kg (GB/T 5009.93-2019).

2.2. Material pre-treatment and powder preparation

Fresh rice samples (cleaned with deionized water) were divided into seven parts i.e., glume, episperm, aleurone, embryo, endosperm, brown rice, and polished rice. The material pre-treatment and powder were prepared as per the following method [19]. Then pulverized into powder and filtered with a 100-grade sifter to obtain the experimental flour [20].

2.3. Determination of selenium content

Selenium content in different grain fractions and rice parts (glume) were identified using method suggested by Ref. [21], using dual channel atomic fluorescence analyzer (RGF-6800, Bo Hui Co., Ltd, Beijing, P. R. China) [22].

2.4. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) analysis

Freeze-dried rice protein sample was dissolved in a sample buffer containing 2 mL 60 mM Tris–HCl (pH 6.8), 25% glycerol (v/v), 0.1% bromophenol blue (w/v), 2% SDS (w/v) and 14.4 mM β -mercaptoethanol (BME). The sample was boiled at 100 °C for 3 min being added to reduce the disulfide bonds of rice protein. Then the treated sample was centrifuged at 6000g for 20 min, and the supernatant was subjected to SDS-PAGE method [23]. The gels were subsequently fixed in 50% ethanol with 10% glacial acetic acid

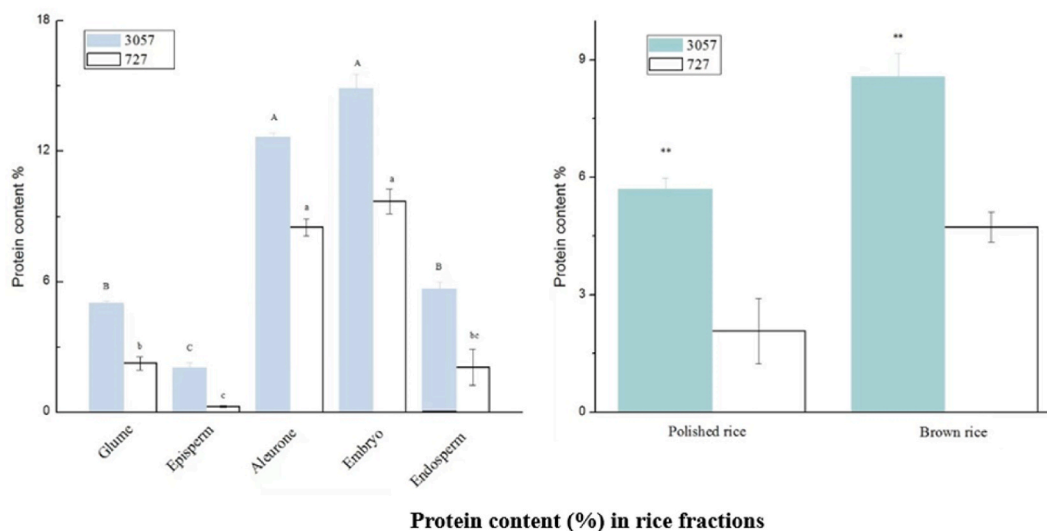


Fig. 1. The protein content (percentage) among different grain fractions in rice genotypes Z3057B and Chenghui 727. Note: The protein content (%) in different parts (glume, episperm, aleurone, embryo, endosperm, polished rice, brown rice). Different capital letters indicate significant differences between different parts in Se-enriched Z3057B rice ($P < 0.01$), different lowercase letters indicate significant differences between different parts in non-Se-enriched Chenghui 727 rice ($P < 0.01$) ** on the other hand indicate significant differences between polished and brown rice ($P < 0.01$). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

for 1 h, stained with 0.29g Coomassie Brilliant Blue R-250 for 2 h. The first protein band and the last protein band were cut into 9 segments (every 2 cm) and compared with the molecular weight calibration (ranging from 10 to 170 KDa). The cut bands were run on the same gel to identify the molecular weights of the protein bands in the stained gels. At the same time, the section is also used for the determination of selenium. After deducting the parallel blank, the selenium content of each fragment was analyzed by atomic fluorescence spectrophotometry [3].

2.5. Data Processing

The experimental data were expressed as the mean \pm standard deviation. DPS statistical software adopted for data analysis and origin 8.0 for plotting graphs. The significant difference among groups was tested by one-way analysis of variance (ANOVA) test, and intergroup differences were compared using least significant difference (LSD) *t*-test at 5% level of probability.

3. Results

3.1. Protein content in different parts of rice

The differences in protein content between the brown rice and the polished rice of Z3057B and Chenghui 727 were highly significant. The protein content percentage was maximal in Z3057B brown rice while minimal in Chenghui 727 polished rice (Fig. 1). Among brown rice different fractions, the protein contents in the embryo, aleurone layer, endosperm, glume and episperm of the two rice cultivars decrease in succession. The difference in protein content of embryo and endosperm for Se-enriched rice (Z3057B) was significant, while that of embryo and aleurone layer was not so obvious. The Protein content in Z3057B embryo accounts for 14.7%, while 1.9% in Z3057B episperm.

3.2. Selenium concentration in different parts of rice

When the selenium contents were observed (Fig. 2), a similar defining pattern as protein content (%) was seen between different rice cultivars for rice fractions (brown rice and polished rice). The total Se content of Se-enriched rice was significantly higher than that of non-Se-enriched rice, similar as protein content. The maximal Se concentrations were observed reside in the embryo up to 0.137 mg/kg, which was significantly different from the selenium content in the other parts of Se-enriched rice Z3057B. However, episperm has the lowest Se concentration which was only 0.023 mg/kg. Se contents of the embryo, aleurone layer, endosperm, glume, and episperm decrease in succession. While the relationship between Se and protein content accumulation in rice fractions was positive, i. e., higher the protein content, higher will be the Se content in various parts of rice grains. The amount of Se that can be retained in polished rice of Z3057B and Chenghui 727 is 0.07 mg/kg and 0.02 mg/kg respectively. A 100 g consumption of polished rice in the diet will provide Se concentration of 7.0 μ g/100g and 2.0 μ g/100g respectively.

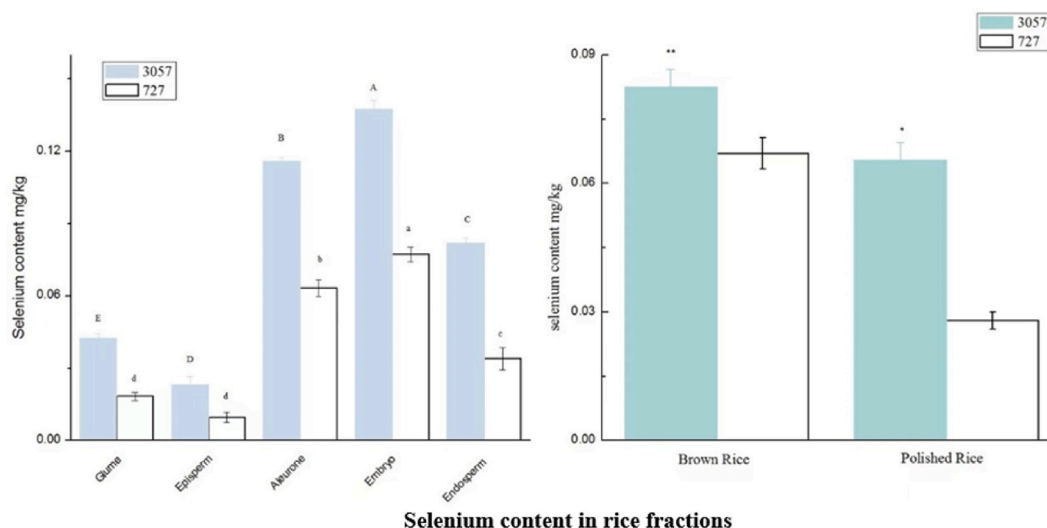


Fig. 2. The selenium concentration among different grain fractions in rice genotypes Z3057B and Chenghui 727

Note: The selenium concentration in different parts (glume, episperm, aleurone, embryo, endosperm, brown rice) of rice grain. Different capital letters indicate significant differences between different parts in Se-enriched Z3057B rice ($P < 0.01$), different lowercase letters indicate significant differences between different parts in non-Se-enriched Chenghui 727 rice ($P < 0.01$) ** on the other hand indicate significant differences between polished and brown rice ($P < 0.01$).

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3.3. Molecular weight distribution of proteins in Se-enriched rice

The protein determination results showed that the various parts of the selenium (Se)-enriched Z3057B and non-Se-enriched Chenghui 727 rice grains vary in protein content. The protein contents vary in the rice fraction layers viz., embryo, aleurone layer, endosperm, glume, and episperm of the two rice cultivars. The total proteins extracted from Se-enriched rice were analyzed by SDS-PAGE (Fig. 3). Quantity one analysis software was adopted for image analysis. According to the molecular weight range of protein, both the synthesized gel and the Se-enriched Z3057B samples were dissected, from which I-VII fragments containing 18 proteins or protein subunit/bands were identified. Results indicated a protein molecular weight range of 13.6–122.6 kDa. The molecular weight and relative content of proteins (%) in each group (Fig. 4) indicated an interesting relationship. Within group I, the molecular weight of each protein was 122.6 kDa. However, the relative percentage of protein content was very low (3%). The sequence was determined for each group separately. For group II, the range was 74.2–97.8 kDa, for group III, it was 48.3–60.8 kDa, 42.7–44.5 kDa for group IV, 23.2–38.8 kDa for group V, 17.6–21.5 kDa for group VI, 13.6–15.2 kDa for group VII. The bandwidth relying molecular weight of 13.6 kDa had the highest protein content, accounting for 40.76% of the total protein content.

The molecular weight distribution of selenoprotein in Se-enriched rice Z3057B was divided into seven groups (Fig. 5). The relative Se and protein content relationship was detected. Selenium is found in all proteins or protein subunits, indicating a significant uneven combination of Se and protein of different molecular weight. The Se concentration reaches a peak of 38.0 $\mu\text{g/g}$ with relative proportion of >50 percent in group VII. While the lowest Se concentration of 4.07 $\mu\text{g/g}$ with relative proportion of 10% was found in group I. This indicated that Se is prone to combine with proteins of low molecular weight i.e., the lower the molecular weight of the protein or protein subunit, the higher the relative content (%) and content of Selenium will be.

4. Discussion

The concentration of Se in food depends on the presence of available forms of Se in the soil and on its uptake and accumulation by plants. Here we investigated the effects of selenium enrichment on the protein content and distribution in different parts of rice, which can provide valuable insights into the nutritional quality of rice. Advanced Selenium biofortified breeding material developed after generations of breeding efforts was selected, which can enhance the reliability and applicability of the findings. At present, the supplementation of Se from external sources is necessary for most Se-enriched products to meet standards. The Se-enrichment in rice by foliar spray of inorganic Se fertilizers such as Na_2SeO_3 [24,25] makes it difficult and risky to ensure Se-enrichment standards. The Se content in products should be in safe range to utilize for humans as too many inorganic Se compounds are harmful to human health and could cause selenosis. To ensure the health and safety of rice products, it is necessary to monitor and select the rice strong in absorbing the Se from soil. After five years of breeding efforts, experimentation and cultivation trials, the Se-enriched rice Z3057B was screened out, with Se concentration in safe range of 0.049 mg/kg, which meets the Chinese national standard of 0.04–0.30 mg/kg for Se enrichment, and meet the daily needs of the human body for Se.

The rice grain is comprised of components viz., glume, episperm, aleurone layer, endosperm, and embryo from the outermost to the innermost cortex (Fig. 6). In current study, the protein and Se contents in the embryo, aleurone layer, endosperm, glume, and episperm

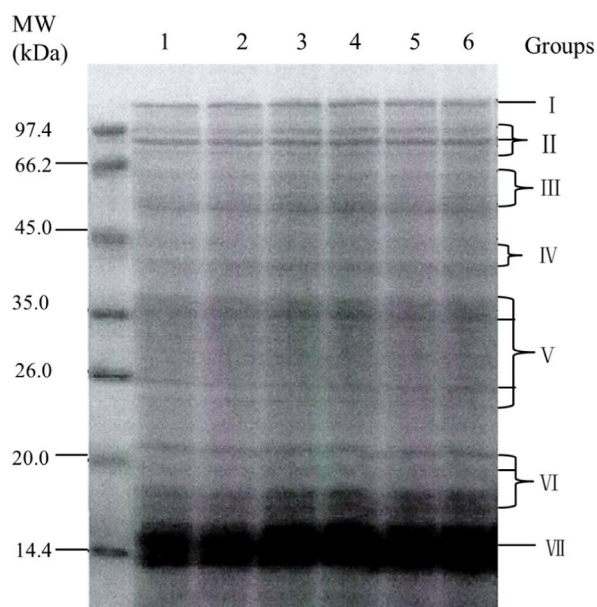


Fig. 3. SDS-PAGE analysis of total proteins extracted from Se-enriched brown rice. MW: Standard molecular weight. Swimlane 1–6 were all total proteins extracted from Se-enriched rice.

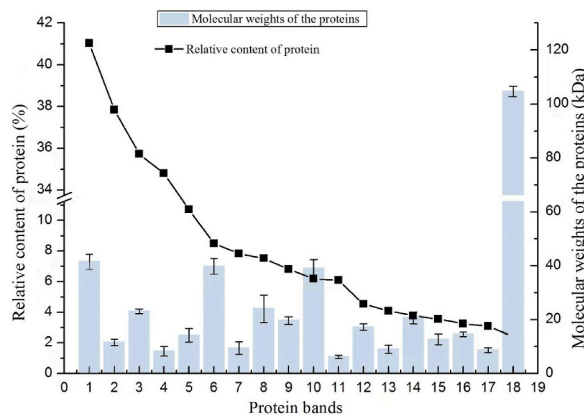


Fig. 4. Protein relative content (%) of each band from the SDS-PAGE.

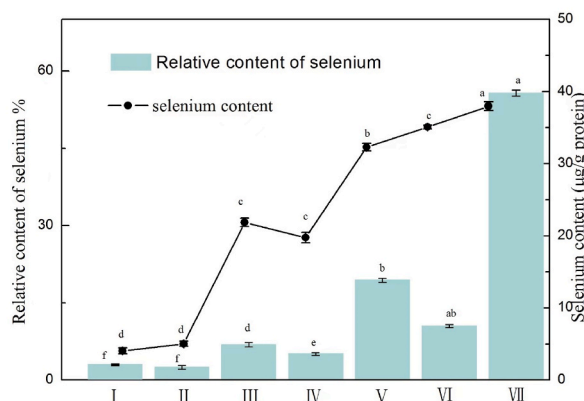


Fig. 5. Relative selenium contents in Se-containing proteins of Se-enriched brown rice. Different letters on the top of column indicate significant differences at $p < 0.01$. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

decreased in succession. A 100 g consumption of polished rice in the diet provides Se concentration of 7.0 $\mu\text{g}/100\text{g}$ (Z3057B) and 2.0 $\mu\text{g}/100\text{g}$ (Chenghui 727). According to Xie's study rice grown in China with protein content ranging between 6.7% and 13.8% [26]. Rice is relatively low in protein content, ranging between 6 g/100g and 8g/100g. The degree of milling determine the protein contents in brown rice [13]. The protein contents dispersion variation in various rice grain fractions indicated embryo and aleurone layer relatively have higher residence of it. Previous studies have shown that brown rice is nutritious compared with polished rice in terms of dietary fiber, vitamins and proteins, while polished rice is predominated by endosperm and embryo [27]. This is consistent with the findings of current research, that is, as for the two rice cultivars (Se-enriched Z3057B and non-Se-enriched Chenghui 727), the Se and protein contents in brown rice are relatively higher than those of polished rice.

Trace elements are abundant in the rice bran, the outer cortex of rice. During the milling and grinding process for brown rice, the loss of trace elements is as high as 84.7% [13]. In the process of milling from brown rice into polished rice, the Se content is inevitably lost somewhat. Therefore, the embryo and aleurone of brown rice are higher in Se content compared with polished rice. Sun [28] have also gained the same conclusion by studying the various parts of rice. Researchers studied Se-enriched edible fungi had showed that the molecular weight of most selenoproteins or protein subunits was 16 kDa at most, and exogenous inorganic Se can combine with all proteins with a molecular weight of 8.71–142.53 kDa [29,30]. The findings of current study were consistent i.e., the proteins of all molecular weights (in Se-enriched rice) combine with Se (SDS-PAGE). The results showed that different parts of rice grains enriched and not enriched in selenium differ in protein content. We concluded that there is a correlation between selenium and protein content in different parts of rice. Interestingly, the Se was prone to combine with micromolecular proteins with the combination amount inversely related to the protein molecular weight. This is probably because of β -hydroxyethanol in the pretreatment of electrophoresis samples, which serve to decompose the disulfide bond of protein into corresponding subunits of low molecular weight. Plant self-comfort has already been established as a pioneering approach to the production of selenium-enriched agricultural products.

Current study is of interest to breeders, stakeholders, persons involved in agricultural research and development and policy makers as it investigates the effects of selenium enrichment on the protein content and distribution in different parts of rice, which can provide valuable insights into the nutritional quality of rice. Further, it uses advanced selenium biofortified breeding material developed after generations of breeding efforts, which can enhance the reliability and applicability of the findings. The detailed experimental

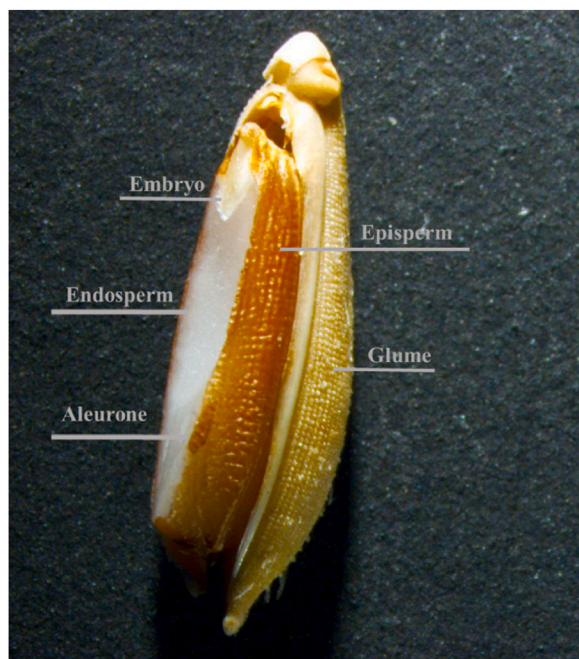


Fig. 6. The grain of selenium-enriched rice.

methodology and data analysis followed, could facilitate the reproducibility and transparency of the study. It has been discovered that selenium binds with proteins of different molecular weights in a regular pattern. Furthermore, the proteins related to selenium-rich ability and related genes localization, combined with various structural identification techniques, the three-dimensional structure of selenium protein could be analyzed i.e., the specific protein concentration values for each layer of rice, the effects of selenium enrichment on other nutritional components of rice, such as vitamins, minerals, and potential health benefits/risks. Future studies could impart significant contribution based on current preliminary research findings Therefore, current study may have prospects in the areas of sustainable agriculture, food security, and public health.

5. Conclusions

The study focuses on enhancing rice's nutritional quality and safety for human consumption. It discovered a link between selenium binding strength and protein structures of varying molecular weights in different rice grain fractions. Selenium content peaked in the embryo of selenium-enriched rice and decreased in other parts. The protein in Z3057B rice predominantly ranged from 13.6 to 122.6 kDa, with selenium binding strength inversely correlated with protein molecular weight. Remarkably, proteins with lower molecular weights, particularly at 13.4 kDa, exhibited the highest selenium binding capacity, constituting 40.76% of the total protein content. A significant portion of selenium, 67.5%, was bound to proteins with a molecular weight of less than 38.8 kDa. The study implies for sustainable agriculture, food security, and public health.

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Data Availability Statement

The data associated with this study was not deposited into a publicly available repository before. All data are included in the manuscript.

Complete ethical statement

All experiments were approved by the Human Ethics Committee of Sichuan Agricultural University (Chengdu, China). All other applicable ethical requirements are carefully observed and followed.

CRedit authorship contribution statement

Rui Zeng: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Yang Su:** Formal analysis, Data curation. **Renwei Huang:** Methodology, Investigation. **Ling Li:** Writing – original draft, Validation. **Muhammad Asif:** Writing – review & editing. **Muhammad Umer Farooq:** Writing – review & editing, Writing – original draft, Formal analysis. **Xiaoying Ye:** Software, Project administration. **Xiaomei Jia:** Writing – review & editing. **Jianqing Zhu:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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