Quick selenium accumulation in selenium-rich rice and its physiological changes in selenium environment

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Abstract

Background: The element selenium (Se) deficiency is recognized as a global health problem. Improving Se accumulation in rice grain is an important issue for human health. But Se is both essential and toxic for plants, with a narrow margin between deficiency and toxicity and the mechanism for Se accumulation and tolerance in selenium-rich rice plants remains unknown. Results: In this study, we investigated the phenotypical, physiological and biochemical changes of selenium-rich rice exposed to different stages of Se concentrations. Results showed that selenium-rich rice can accumulate more Se from the root in low selenate environment comparing with selenium-free normal rice. Besides, excessive selenate caused phytotoxic effects on selenium-rich rice plants by inducing chlorosis, dwarfness, reduced antioxidant contents, and exacerbating oxidative stress. Furthermore, both phosphate transporter OsPT2 and sulfate transporters OsSultr1;2 can contribute to selenite uptake in rice. Conclusions: Based on the results, selenium-rich red rice is more sensitive to Se and the best Se concentration for selenium-rich rice is 20μM in the root environment. Such findings can be used to direct and evaluate the toxic effects of Se contamination on selenium-rich rice in the field. To draw a sound conclusion, long-term field trials are still required, including risks and benefits analysis for various management strategies.

Background

Selenium (Se) is an indispensable micronutrient for humans and animals. Studies have shown that selenium supplementation enhances the ability to scavenge free radicals and can coordinate the immune response and help delay the aging of the immune system [1–3]. Selenium resists cellular senescence and death by resisting peroxides and scavenging free radicals, thereby reducing or delaying the formation of lipofuscin [4]. However, its
essentiality for plants has not yet been established [5]. Although the human demand for Se is not high, the abundance of Se is very low in the Earth’s crust. Thus, Se deficiency is still recognized as a global health problem that needs to be addressed [6].

For plants, some scientists believe that selenium is a beneficial element because it has many effects on plant growth and development, including regulating plant photosynthesis and respiration, reducing free radicals damage to plants, enhancing plant stress resistance, and alleviating the toxicity of heavy metals to plants [1, 7]. At the same time, it can increase chlorophyll and carotenoid content in plant leaves, reduce damage caused by ultraviolet-induced oxidative stress [8], stimulate plant growth, and enhance plant tolerance or stress resistance to abiotic or biotic stresses [9]. Low selenium concentration is believed to be beneficial to plant growth and development, while high selenium concentration may be toxic to plants [10].

Rice (Oryza sativa L.), as one of the most important food crops, is the staple food source for more than half of the world’s population and contributes 55%-80% to the total calorie of people [11]. Its selenium content is closely related to human selenium nutritional status. Lack and excess of selenium will affect human health [7, 12]. Moreover, food health is a burning issue in the world. Avoiding selenium deficiency and toxicity, it is important to monitor and optimize crop selenium quality and concentrations, though it can vary greatly between different crops and regions [7, 13]. Therefore, it is necessary to study the effect of sodium selenate on selenium-rich rice.

At present, the research on selenization of rice mainly focuses on two aspects: one is to improve the selenium content in rice by applying exogenous sodium selenate from the perspective of physiological cultivation; the other is to breed selenium-rich parents according to the genetic differences among varieties [1, 5, 14, 15]. Studies have shown that genotype essentially determines the difference in Se uptake and enrichment in rice
Besides, some scientists reported that silicon (Si) transporter protein \( Lsi1 \) (\( OsNIP2;1 \)) in rice is permeable to selenite [17]. What’s more, selenite and phosphate (Pi) share similar uptake mechanisms, so Pi transporters, such as rice \( OsPT2 \), catalyze the uptake of selenite [18]. In \textit{Arabidopsis thaliana} and \textit{Stanleya pinnata}, sulfate transporter \textit{SULTR1;2} plays a central and specific role in selenate sensitivity [5, 19, 20]. In addition, cadmium (Cd) has antagonistic effects on selenium, as disclosed by previous work [21]. Thus, it appears that the uptake of selenium by plants is affected by various elements transporters. However, selenium comes from the soil, and selenium in naturally selenium-rich rice grains indicates that selenium-rich rice has a strong ability to absorb and enrich selenium, but its mechanism is rather limited.

Therefore, the selenium-rich red hybrid rice \( Z2057A/CR727 \) and selenium-free rice \( CR727 \) obtained by heterosis were used as experimental materials to explore the physiological effects of sodium selenate on them, by using different selenium concentration levels i.e., how selenium affects the physiological growth of selenium-rich red hybrid rice and Se free rice seedlings. Furthermore, the selenium transport behavior will be accessed in both rice types, either selenium-rich rice can absorb and transport selenium to leaves. Rice physiology, anthocyanin, and selenium accumulation in different parts and biochemical activities will be accessed at 48 hours and 14 days, helps provide a better understanding of different material behavior. The optimum level of sodium selenate could be recommended for safe selenium range in plants.

\textbf{Methods}

2.1. Plant materials, growth conditions and treatments

Healthy selenium-free rice (\textit{Oryza sativa} L.) \( CR727 \) and selenium-rich red hybrid rice \( Z2057A/CR727 \) seeds were collected with the consent of the Demonstration Base for International Science & Technology Cooperation of Sichuan Province, Rice Research
Institute, Sichuan Agricultural University, Chengdu, Sichuan, China. Rice seeds were surface sterilized with NaOCl [1% (v/v)] for 20 min and washed 5 times with double distilled water (ddH₂O) followed by imbibition for 48 hours. For germination, the seeds were placed on plastic nets, floating on distilled water (dH₂O) and kept in an incubator (37 ± 1°C) under dark for 72 hours. Uniformly germinated seeds were sorted and cultivated in a growth 1000ml plastic chamber with half-strength Kimura B nutrient solution and housed under a light-dark cycle of 12 h:12 h at 25 ± 2 °C, using a hydroponic solution [17]. The nutrient solution was adjusted to a pH of 5.5 using 1 M NaOH and renewed at 3 days interval [16]. After 12 days, healthy seedlings were exposed to environmentally toxic Se stress by supplying 10, 20, 40 and 80 μM sodium selenate [Na₂SeO₄, referred to as Se hereafter] in the nutrient solution [10]. The control and Se-treated seedlings were grown for 14 additional days in the above-stated conditions [22]. The fully expanded second leaves of rice seedlings were harvested to determine growth-related parameters associated with physiological and biochemical responses. Three independent replications of each treatment were used in determining each parameter.

2.2. Measurements of leaf water status

Leaf relative water content (RWC) was calculated based on fresh weight (FW), turgid weight (TW) and dry weight of leaves samples. Approximately 0.1 g fresh leaves were detached and immediately weighed as the FW, then soaked in de-ionized water for 24 hours at 4 °C in the dark and weighted as TW. Leaf samples were then dried in an 80 °C oven for at least 72 hours prior to being weighted for DW. The RWC was calculated as (FW-DW)/(TW-DW) × 100% [23].

2.3 Measurements of physiological parameters

Total leaf chlorophyll (Chl) content analysis, fresh leaves (0.1 g) were immersed in 10 ml
of dimethyl sulphoxide in the dark for 48 hours, and then the leaf extract was measured at 663 and 645 nm with a spectrophotometer [24]. According to the manufacturer’s instructions of the kits from Nanjing Jiancheng Bioengineering Institute (Nanjing, China), superoxide dismutase (SOD) and methane dicarboxylic aldehyde (MDA) were determined by the Shimadzu UV-visible spectrophotometer on path length cuvettes of 1 cm (T6S, Puxi, Co., Ltd, Beijing, P. R. China).

2.4 Histochemical Analysis
After 14 days of treatment, rice leaves were collected for histochemical analysis. 3,3’-diaminobenzidine (DAB, 1.0%) staining was performed to detect hydrogen peroxide ($H_2O_2$) accumulation [25]. To observe the accumulation of reactive oxygen species (ROS) in leaves, nitroblue tetrazolium (NBT, 0.1%) was performed as previously described [25].

2.5 Measurements of selenium and anthocyanins content
To determine the Se content, we used the atomic fluorescence spectrophotometer (RGF-6800, Bo Hui Co., Ltd, Beijing, P. R. China) as previously described [26, 27]. To determine the anthocyanins content, we used the Shimadzu UV-visible spectrophotometer according to the previously described [11].

2.6 Relative genes expression analysis
According to the qRT-PCR method [3], mRNA of two-week treatment rice seedling roots was extracted by using the TRI pure reagent kit (Aid Lab). Primers used in these assays synthesized by Qingke Company (Qingke Zixi Co., Ltd., Chengdu, China) are listed in Table 1, and the expression levels were normalized to those of the Actin1 and EF1α as indicated.

TransScript® All-in-One First-Strand cDNA Synthesis Super Mix for qPCR (One-Step gDNA Removal) kit (Transgen, Beijing, China) and Universal SYBR® GREEN qPCR Master Mix (2X) were used to perform qRT-PCR on CFX Connect™ Flex Real-Time PCR System (BIO-RAD
The conditions of thermos cycling were 40 cycles of 95°C for 15 s, 55°C for 15 s, and 72°C for 20 s. The $2^{-\Delta\Delta CT}$ method was used to calculate the expression levels of target genes[28].

2.7 Statistical Analysis

All analyses were performed in triplicate. Data expressed as mean ± standard error (SEM). One-way ANOVA was carried out with multiple comparisons using Duncan’s test to detect significant differences among the five groups with different concentration sodium selenate treatments at $p \leq 0.05$. All statistical analysis was performed with the SPSS 24.0 statistical package (SPSS Inc., Chicago, IL, USA).

Results

3.1. Physiological differences and selenium accumulation trend for selenium-rich red hybrid rice and selenium-free rice

Fig. 1 (b) shows the root length for both rice types under investigation. Compare with the control group, 40μM selenium environment can enhance root length of normal rice CR727. Meanwhile, sodium selenite can not promote the root elongation of selenium-rich red rice Z2057A/CR727. On the other hand, the highest selenium concentration 80μM significantly decreases the root length of both rice types.

The same principle was observed in Fig. 1 (c) for leaf length. The selenium-rich red rice hybrid was shorter than its parent for leaf length. Overall the selenate treatment can’t enhance the leaf length in both rice types. At 80μM, the growth of leaves was inhibited in both rice types. It was concluded that the high concentration of 80μM was not suitable for
leaf and root growth in both rice types. It was observed that the treatment levels of 10μM, 20μM, and 40μM significantly enhanced the chlorophyll contents for rice under investigation (Fig.1d). An elevated level of selenate treatment at 80μM reduces the chlorophyll contents to the same as control. The physiological differences between genotypes can be observed in Fig. 1 (a).

Table 2 shows selenium content in roots, stems, leaf top and main leaves for both genotypes. It was observed that the selenium contents increased significantly with the increase of sodium selenate concentration in different plant parts. A similar ascending trend for selenium accumulation was observed for selenium-free rice and selenium-rich rice for root and stem parts while the trend for leaf tips and all leaf produce was different for material under investigation. At 20μM and 40μM selenate treatment, the accumulation of selenium was maximum in the stem; leaf tips and all leaf produce while a high concentration of 80μM decreased the selenium accumulation. This indicated that the selenium-rich red rice hybrids were more sensitive to Se uptake and selenium concentration significantly changed under low selenate concentration than selenium-free rice.

3.2. Effects of sodium selenate on the growth of selenium-rich red hybrid rice and selenium-free Rice

When compared the morphology of rice material under different treatment levels, we observed that the high concentration of sodium selenate limits the height of selenium-rich rice both at 48 hours and 14 days (Fig. 2). When the material was compared for plant height the selenium-free rice was found to have more height for all treatment levels than selenium-rich rice. However, after 48 hours of treatment, the leaves color was green, and
the leaves were healthy (Fig. 2a). After 14 days of treatment with different selenate concentration, the leaves length of selenium-rich hybrid rice and selenium-free rice increased than 48hr observations while the leaves were still green in color (Fig. 2b). Sodium selenate concentration of 80μM proves dangerous for the material under study. The leaf color of selenium-rich red hybrid rice and selenium-free rice changed to yellow-green, the length of roots and leaves shortened with the decreased chlorophyll contents, Hence, the number of dead leaves increased significantly at the 80μM level.

3.3. Effects of sodium selenate on the biochemical activity and relative water contents

According to the obvious lesion mimic spots phenotype in rice leaves, we performed NBT and DAB staining to visualize superoxide and $H_2O_2$ accumulation, respectively. Fig. 3 (a, b) indicated that NBT and DAB-stained were observed in the high Se concentration groups but not low Se concentration leaf blade, whereas there were rarely observed in the 20μM Se group for selenium-rich red rice. Starting from 40μM selenate, Se-induced superoxide and $H_2O_2$ accumulation in selenium-rich red rice leaves. For selenium-free general rice CR727, we can see a little lesion spot in 80μM.

The behavior for different enzymatic activity (SOD, MDA) was absolute at low sodium selenate treatment levels. SOD enzymatic activity found increased at 10μM and 20μM as compared to control while the corresponding MDA contents were found reduced (Fig. 3c, d). The behavior at high (40μM and 80μM) sodium selenate treatment levels was abnormal for SOD and MDA activity, clearly indicated the harmful effects of a high dose of selenate concentration. 40μM selenate treatment drastically reduces the SOD contents while 80μM sodium selenate treatment level was stress itself, it increases the MDA contents in the
plant while the SOD activity was also found increased. The high SOD activity at 80μM level selenate treatment completely seems helpless to reduce the MDA, proves it as stress for the plant instead of a benefit. These results were favored by other parameters as well in the present study. This indicates that low level of selenate concentration can help the plant and reduce the formation of MDA and the degree of membrane lipid peroxidation can also be significantly reduced.

When we observed the relative water contents (RWC) under different sodium selenate treatment levels, we found that they were maximum at low selenate concentration of 10μM and 20μM (Fig. 3e). The high level seems to reduce the RWC in both rice types while the water contents of selenium-free rice seems to be high then selenium-rich rice for all the treatment levels. Low-concentration of sodium selenate seems beneficial to both selenium-rich red hybrid rice and selenium-free rice to increase its relative water content and enhance its ability to resist stress. Moreover, selenium-free rice was more resistant to medium-high (40μM and 80μM) sodium selenate stress than selenium-rich red hybrid rice.

3.4. Effects of sodium selenate on gene transcription of selenium uptake-related transporters in rice seedling roots

To further investigate the physiological function of sodium selenate in selenium uptake by selenium-rich rice roots, we examined the effect of sodium selenate on the gene transcription of several Se uptake-related transporters including a phosphate transporter (OsPT2), a Si influx transporter (OsNIP2;1), a sulfate transporter (OsSultr1;2), and a Cd relative transporter (CAL1) at sodium selenate supply using real-time quantitative fluorescent PCR. For both rice, the expression of OsPT2 increased in the roots when sodium selenate was applied in comparison to the seedlings grown in half-strength Kimura B nutrient solution without Se (Fig. 4a). Notably, for selenium-rich rice Z2057A/CR727, we observed a sharp increase of around 4-fold in OsPT2 transcription in 20μM dose compared
to without Se environment. Nevertheless, the transcription of OsNIP2;1 in roots exhibited an elusory trend under sodium selenate treatments (Fig. 4b). For the normal rice CR727, in the highest dose of Se (80μM), the expression level of OsNIP2;1 was abruptly decreased but the gene expression level has no significant variation in other lower dose Se environment. What’s more, application of Se to rocket plants led to significant changes in the transcript abundance of roots high-affinity sulphate transporter gene OsSultr1;2. A clear Se dose-dependent increase was observed for OsSultr1;2 expressions in both rice, while OsSultr1;2 transcriptions was up-regulated to similar levels by 10 to 80 μM Se (Fig. 4c). For the gene transcription of Cd transporter, in normal rice CR727 roots, the expression of the Cd transporter CAL1 had a low-affinity in Se-dose dependent (Fig. 4d). In contrast, CAL1 transcription in selenium-rich rice Z2057A/CR727 roots was appreciably reduced by low Se-dose concentrations. These results suggested that sodium selenate enhanced root selenium uptake and transport by up-regulated gene transcription of OsPT2 and OsSultr1;2 in rice seedling roots.

Discussion

Worldwide, more than 85% of the rice is white-hulled while other types have colored hulls, mainly purple, black, and red [29]. In Asia, especially in China, Japan, South Korea and many countries in Southeast Asia, colored rice is consumed over a long time [30]. Compared with conventional rice, colored rice has high nutritional value. Many studies have shown that colored rice and its extracts have high antioxidant activity and free radical scavenging ability in vitro and in vivo experiments [11, 31]. In this experiment, the seeds of selenium-rich red hybrid rice Z2057A/CR727 and selenium-free rice CR727 were selected as experimental materials (Fig. 5a). The physiological effects of different sodium selenate concentrations on the two rice varieties were studied. The results showed that sodium selenate concentration in a certain range was beneficial to the growth of the two
kinds of rice, while the selenium-rich red rice hybrid rice was more sensitive to the change of sodium selenate concentration.

Fig. 5 shows that the selenium content and anthocyanin content in seeds and brown rice of selenium-rich red hybrid rice were higher than those of their parents due to their heterosis and the length and weight of grain were between the expression of both parents. Anthocyanins, which belong to flavonoids, are water-soluble natural pigments widely distributed in plant stems, leaves, flowers and fruits [11, 32]. Anthocyanins have strong antioxidant activity, can reduce and eliminate the effects of free radicals [33]. Under different sodium selenate concentrations, low selenate level tended to promote the growth of roots and leaves of two rice varieties, while high selenate tended to inhibit their growth [34]. SOD is a major antioxidant enzyme related to the scavenging of reactive oxygen species, which maintains the balance of active oxygen metabolism and protects membrane structure [35]; MDA is one of the final products of peroxidation of unsaturated fatty acids in phospholipids and is the cause of cell membrane damage [36]. It can be seen from Fig. 3(a)(b) that the effect of selenate on SOD activity and the content of MDA are not simply increasing and decreasing. Only when selenate concentration was appropriate (low levels), SOD activity of two rice varieties seems to be better and MDA content found reduced. However, the activity of SOD increased abruptly under high selenate concentration (80μM) with elevated MDA content. The reason may be that high concentration of sodium selenate has produced selenium toxicity to both rice, while the plant itself activates the activity of SOD to resist selenate stress. This problem is not clear at present and needs further investigations.

As the previous work reported, sulphur replete Arabidopsis mutants sultr1;2, lacking SULTR1;2 but not those lacking other sulphate transporters, take up less selenate and exhibit greater tolerance to selenium in the rhizosphere than wild-type plants [19, 20],
and the expression of genes encoding *SULTR1;2* generally increases in plant when tissue Se concentrations rise [5], which is in accordance with our result. Similarly, the expression of *OsPT2* increases in roots of plants lacking sufficient phosphorus and results in a greater capacity for selenite uptake [37], and rice mutants lacking *OsPT2* take up significantly less selenite than wild-type plants [18]. Zhao et al. believed that when defect of Si influx transporter *OsNIP2;1* resulted in a significant decrease in the Se concentration of the shoots and xylem sap when selenite was given, but there was no difference in the Se concentration between the wild-type rice and mutant of *Osnip2;1* when selenate was supplied [17]. For selenium-rich red rice Z2057A/CR727, we observed the expression level of *OsNIP2;1* was increased in low Se conditions but when the expression decreased in higher Se concentrations. So *OsNIP2;1* may not be the key factor for the Se sensitivity of selenium-rich red hybrid rice. What’s more, it was reported that Cd relative transporter *CAL1* is expressed preferentially in root exodermis and xylem parenchyma cells which acts by chelating Cd in the cytosol and facilitating Cd secretion to extracellular spaces, hence lowering cytosolic Cd concentration while driving long-distance Cd transport via xylem vessels [38]. Thus, we investigated the *CAL1* expression level of selenium-rich rice in selenium environment. According to our result, the expression of *CAL1* has no obvious variation in normal rice CR727 while a little decreased in low Se-dose concentration of selenium-rich rice. It means *CAL1* may do not appear to affect selenium accumulation in rice roots. In addition, it was reported that NRT1.1B, a member of rice peptide transporter (PTR) family, holds great potential for the improvement of Se concentrations in grains by facilitating SeMet translocation, and the findings provide novel insight into the breeding of selenium-rich rice varieties [9]. Roots of Se-hyperaccumulator species have constitutively high expression of these genes, which might account for their large selenate uptake capacity [39].
Different rice varieties have different selenium accretion trend, and the selenium stress treatment of different rice also determines its accumulation pattern. The order of selenium content in different parts of rice was in the order root>leaf>stem as the previous reported [5, 18]. But the apparent trend for selenium accumulation seems to be different under different treatment levels. In the present investigation, the trend was more obvious in the following order: leaves>stem>root depending on the concentration of selenite treatment. selenium-rich red hybrid rice seems to have stronger selenium absorption and translocation ability than selenium-free rice. The content of selenium accretion mainly related to translocation efficiency, as the model of selenate influence on selenium-rich red hybrid rice (Fig 6). The physiological dynamics of selenium-rich hybrid rice did not differ significantly under different selenate concentrations, but it was more beneficial to selenium-rich hybrid rice under low selenate concentration treatment levels (10μM and 20μM), while high selenate concentration level proved as selenium hypertoxicity stress to the plants, which was similar to the previous studies [40]. However, the selenium content of selenium-free rice could increase significantly by increasing selenate concentration, which was consistent with the previous results [10, 27]. selenium-rich hybrid rice was more sensitive to changes in selenate concentration treatment.

Conclusions
This study demonstrated that selenium-rich rice is more sensitive to Se application. It can accumulate more Se from the root in low selenate environment while at high concentration of selenite application the effects were inverted. Besides, excessive selenate can cause phytotoxic effects on selenium-rich rice plants by inducing chlorosis, dwarfness, reduced antioxidant contents, and exacerbating oxidative stress. In addition, we conclude that selenite enhanced gene transcription of phosphate transporter OsPT2 and sulfate transporters OsSultr1;2 to improve the uptake of Se. Meanwhile,
The best Se concentration was 20μM for selenium-rich red rice root environment. Such findings can be useful to estimate the direct toxic effects of Se contamination on selenium-rich rice in the field. To draw a sound conclusion, long-term field trials are still required, including risks and benefits analysis for various management strategies.

**Abbreviations**

Se: selenium; Pi: phosphate; Cd: cadmium; Si: silicon; ddH$_2$O: double distilled water; Chl: chlorophyll; SOD: superoxide dismutase; MDA: methane dicarboxylic aldehyde; DAB: 3,3’-diaminobenzidine; H$_2$O$_2$: hydrogen peroxide; ROS: reactive oxygen species; NBT: nitroblue tetrazolium; qRT-PCR: Quantitative reverse transcription-PCR

**Declarations**

**Author’s Contributions**

JZ and YL conceived the project and designed the experiments; YL, MUF, ZT, YZ, TZ, HLA, XY, XJ, and RZ performed the experiments; YL and MUF analyzed the data; YL, MUF, and YS finalized the manuscript. All authors have read and approved the manuscript.

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**Availability of data and materials**

The data sets supporting the results of this article
are included within the article.

Ethics approval and consent to participate.

The rice samples were collected with the consent of the Demonstration Base for International Science & Technology Cooperation of Sichuan Province, Rice Research Institute, Sichuan Agricultural University, Chengdu 611130, Sichuan, China. No other permissions were necessary to collect these samples.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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Tables

Table 1. Primer sequences for qRT-PCR
| Gene                  | Primer sequences                  |
|-----------------------|-----------------------------------|
| Actin1 (Os03g0718100) | FW: TCCATCTTGCCATCTCTG          |
|                       | RV: GTACCCGCATCAGGCATCTG          |
| EF1α (Os03g08010)    | FW: TTTCACTCTGGTGTAAGCAGAT       |
|                       | RV: GACTTCCCTTCAGATTCATCGTAA      |
| OsPT2 (Os03g05640)   | FW: AAACCTCTCGGTATGCTCATG        |
|                       | RV: ATGTTTATGACATCAGCTG          |
| OsNIP2;1 (Os02g51110) | FW: ACATCCAAAGTGATAGGACG        |
|                       | RV: ACACAAAGACGTCGAGTAGT         |
| OsSultr1;2 (Os03g0195500) | FW: TCAAAAGAAGACCGCTAGATT  |
|                       | RV: GCAATTTCAAGGAAGCCTTAAA       |
| CAL1 (Os02g0629800)  | FW: AGTCGCGTTCTCTCTTGT          |
|                       | RV: GTACCCGCATCAGGCATCTG          |

Table 2. Selenium content of roots, stem, main leaves and leaf top in selenium-rich hybrid rice and selenium-free rice after 14 days treatment.

| Selenium content (mg/kg) | Root | Stem | Leaf | Leaf top |
|--------------------------|------|------|------|----------|
| CR727                    | Z2057A/CR727 | CR727 | Z2057A/CR727 | CR727 | Z2057A/CR727 | CR727 | Z2057A/CR727 |
| 0                        | 0.024<sup>e</sup> | 0.068<sup>e</sup> | 0.032<sup>e</sup> | 0.078<sup>d</sup> | 0.036<sup>d</sup> | 0.084<sup>e</sup> | 0.027<sup>e</sup> | 0.07<sup></sup> |
| 10μM                     | 1.243<sup>d</sup> | 1.528<sup>d</sup> | 5.243<sup>d</sup> | 8.528<sup>c</sup> | 4.327<sup>c</sup> | 10.498<sup>d</sup> | 3.253<sup>d</sup> | 7.36<sup></sup> |
| 20μM                     | 2.984<sup>c</sup> | 3.682<sup>c</sup> | 8.984<sup>c</sup> | 12.283<sup>b</sup> | 7.566<sup>b</sup> | 20.363<sup>a</sup> | 4.358<sup>c</sup> | 12.75<sup></sup> |
| 40μM                     | 5.547<sup>b</sup> | 5.893<sup>b</sup> | 10.546<sup>b</sup> | 13.896<sup>a</sup> | 11.383<sup>a</sup> | 18.652<sup>b</sup> | 6.355<sup>a</sup> | 10.69<sup></sup> |
| 80μM                     | 6.582<sup>a</sup> | 7.491<sup>a</sup> | 11.583<sup>a</sup> | 12.493<sup>b</sup> | 11.159<sup>a</sup> | 14.351<sup>c</sup> | 5.850<sup>b</sup> | 9.49<sup></sup> |

Lowercase letters (a, b, c, d and e) on the right of the data indicate the statistical significance between different groups according to Duncan's test (<i>p</i> < 0.05).

Figures
Figure 1

Root length (a), leaf length (b), chlorophyll (c), seedling phenotype (d). Bar=5cm, data are mean values ± SE (n = 10, a, b; n=3, c). Different uppercase and lowercase letters indicate significant differences (p < 0.05)
Figure 2

Phenotypes of selenium-rich red hybrid rice and selenium-free rice at 48 hours (a) and 14 days (b) after different concentrations of sodium selenate treatment, bar=5cm.
Nitroblue tetrazolium (NBT) staining of superoxide (a) and diaminobenzidine (DAB) staining of hydrogen peroxide (H2O2) (b) and the effect of different selenium concentrations on reactive oxygen species (ROS), SOD activity (c), MDA content (d) and RWC percentage (e) of selenium-rich hybrid rice and selenium-free rice. Data are mean values ± SE (n = 3) and different uppercase and lowercase letters indicate significant differences (p < 0.05).
Expression profiling by real-time RT-PCR of phosphate transporter OsPT2 (a), Si influx transporter OsNIP2;1 (b), and sulfate transporters OsSultr1;2 (c) and Cd relative transporter CAL1 (d) in the roots of rice seedlings. The expression levels were normalized by Actin1 gene. Data are mean values ± SE (n = 6).
Figure 5

The relationship of CR727 and Z2057A/CR727 (a) and comparison of agronomic traits grain length (b), the weight of 1000 grain (c), anthocyanin (d) and selenium (e) contents in brown rice and the seed and brown rice of this experiment material (f). Data are mean values ± SE (n =10, b; n=3 d, e) and different uppercase and lowercase letters indicate significant differences (p < 0.05)
Figure 6

Selenate uptake and assimilation in selenium-rich red hybrid rice Z2057A/CR727.

Selenate is taken up from the soil and fast response in root and accumulated in leaves with the dramatically increasing by OsPT2 and OsSultr1;2 in roots.