**LUBIA DA SILVA TEIXEIRA**

# SELENIUM UPTAKE AND THE NUTRITIONAL QUALITY OF RICE (Oryza sativa L.) GRAINS ARE AFFECTED BY NITROGEN AVAILABILITY

Dissertation presented to the Universidade Federal de Viçosa, as part of the requirements of the Graduate Program in Plant Physiology, to obtain the title of Magister Scientiae.

Advisor: Dimas Mendes Ribeiro

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# RESUMO

TEIXEIRA, Lubia da Silva, M.Sc, Universidade Federal de Viçosa, outubro de 2020. **A absorção de selênio e a qualidade nutricional dos grãos do arroz (Oryza sativa L.) são afetadas pela disponibilidade de nitrogênio.** Orientador: Dimas Mendes Ribeiro.

O selênio (Se) e o nitrogênio regulam o metabolismo primário em plantas de arroz. No entanto, faltam informações sobre como a disponibilidade de nitrogênio afeta a absorção e o acúmulo de Se nos grãos de arroz. Assim, o objetivo do trabalho foi avaliar como o efeito combinado do Se e do nitrogênio podem afetar a produção e a composição nutricional dos grãos de arroz. Não foi observado efeito significativo do Se nas concentrações dos pigmentos, A, $g_s$, $C_i/C_a$ e E na produção de grãos independente do fornecimento de nitrogênio. No entanto, o tratamento com Se combinado ao nitrogênio aumentou a expressão de OsSULTR1.2 e OsPT2 na raiz mais do que o fornecimento de nitrogênio sozinho, enquanto o aumento de OsNIP2.1 foi independente do fornecimento de nitrogênio. Porém, o aumento do suprimento de nitrogênio elevou a expressão relativa de OsNRT1.1B, um transportador de influxo de nitrato, potencializando a translocação de Se da raiz para a parte aérea das plantas tratadas com Se, melhorando o teor de Se nos grãos. Foi observado também um aumento significativo nos teores de N, P, S e Fe em grãos de plantas de arroz tratadas com Se cultivadas em condições limitantes de nitrogênio, enquanto o teor de Ca aumentou independentemente do fornecimento de nitrogênio. Entretanto, o Se tendeu a reduzir as concentrações de Cu e Zn e aumentar o teor de Mg e Mo nos grãos de plantas cultivadas em 16 mM e 24 mM de nitrogênio em comparação com plantas limitadas por nitrogênio. Além disso, existiu uma interação sinérgica entre o nitrogênio e o Se no teor de proteína total, uma vez que a fração de glutelina foi acentuadamente elevada nos grãos das plantas tratadas com Se. Ademais, o Se aumentou o teor de aminoácido total no grão de plantas cultivadas em condição limitada e em 16 mM de nitrogênio, em relação às plantas tratadas com suprimento comparável de nitrogênio. No entanto, o efeito do aumento da concentração de nitrogênio nas concentrações de açúcar solúvel e amido foi independente do fornecimento de Se. Assim, a combinação de Se a concentrações de nitrogênio teve um efeito positivo nos níveis calóricos dos grãos, mas um efeito negativo na concentração de uma gama de minerais (Fe, Zn, Cu e Mn).

Palavras-chave: Expressão gênica. Fertilização com selênio. Produção de arroz. Suprimento de nitrogênio.

# ABSTRACT

TEIXEIRA, Lubia da Silva, M.Sc, Universidade Federal de Viçosa, October, 2020. **Selenium uptake and the nutritional quality of rice (Oryza sativa L.) grains are affected by nitrogen availability.** Advisor: Dimas Mendes Ribeiro.

Selenium (Se) and nitrogen regulate primary metabolism in rice plants. However, information on how nitrogen availability affects Se uptake and accumulation in rice grains is hitherto lacking. Thus, the aim of the work was to evaluate how the combined effect of Se and nitrogen can affect the rice production and grain nutritional composition. Were observed no significant effect of Se on concentrations of pigments, A, $g_s$, $C_i/C_a$ and E regardless of nitrogen supply. Although Se had no effect on grain production, Se treatment combined with nitrogen treatment increased the expression of OsSULTR1.2 and OsPT2 in root more than nitrogen supplies alone, while the increase in OsNIP2.1 was regardless of nitrogen supply. However, the increase in nitrogen supply, improving the relative expression of OsNRT1.1B, a nitrate uptake transporter, enhancing the translocation of Se from the root to shoot of plants, improving the Se content in grains of rice. Our results also showed a significant increase in concentrations of N, P, S and Fe in grain of rice plants treated with Se grown in nitrogen-limiting conditions while the Ca concentration increased regardless of nitrogen supply. On the other hand, Se tended to reduce concentrations of Cu and Zn and increased concentration of Mg and Mo in grain of plants grown in 16 mM and 24 mM nitrogen compared with nitrogen-limited plants. Furthermore, there was a synergistic interaction between nitrogen and Se in the content of total proteins in the grains, since the proportion of the glutelin fraction was markedly elevated in the grains of Se-treated plants. In addition, Se led to a significant increase of total amino acid in grain of plants grown in nitrogen-limited condition and in 16 mM nitrogen when compared with plants grown at a comparable nitrogen supply. However, the effect of increased nitrogen concentration on the concentrations of soluble sugar and starch was independent of Se supplied. Therefore, the combination of Se plus nitrogen concentration had a positive effect on caloric levels of grain, but a negative effect on the concentration of a range of minerals (Fe, Zn, Cu and Mn).

Keywords: Gene expression. Selenium fertilization. Rice yield. Nitrogen supply.

# LIST OF SYMBOL AND ABBREVIATIONS

Se = selenium

A = net $CO_2$ assimilation rate.

$g_s$ = stomatal conductance.

$C_i$/Ca = intercellular-to-atmospheric $CO_2$ ratio

E = transpiration rate.

PAR = photosynthetically active radiation.

N = nitrogen

P = phosphorus

K = potassium

S = sulphur

Ca = calcium

Mg = magnesium

Mn = manganese

B = boron

Mo = molybdenum

Cu = copper

Zn = zinc

Fe = iron

# SUMMARY

## 1. Introduction

Selenium (Se) is an essential element for human health due to its antioxidant properties (Fairweather-Tait et al, 2011). In this context, selenium plays key roles in the immune system, reducing viral infections and inflammatory conditions in the human body (Rayman, 2012). The recommended average daily intake of selenium in adult diet is 55 μg (White, 2016). Deficiency of this element is associated with many diseases (Fordyce 2013), affecting approximately one billion people worldwide (Zhang et al, 2019). This is mainly due to the ingestion of food with low Se content, resulting from its low concentration in the soil for absorption by plants (White 2016; Reis et al 2018). In this sense, supplementation with Se in crops considered basic in human nutrition, such as rice, is an interesting alternative to mitigate the effects of suboptimal consumption of Se by the world population (Lidon et al, 2009).

Although Se is not an essential nutrient for plants, it is considered a beneficial element (El Mehdawi and Pilon-Smits 2012). For example, Se at low concentration may increase biomass production of rice plants by regulating net $CO_2$ assimilation rate (A) and antioxidant enzymes (Zhang et al 2014b, Deng et al 2017). Plant roots obtain Se from the soil predominantly as selenate and selenite (Terry et al 2000). Selenium is chemically similar to sulfur, therefore, most plant species can absorb and metabolize this element through the same sulfate assimilation pathway (El Mehdawi et al 2014). The inorganic forms of Se, such as selenate and selenite, are assimilated to selenocysteine and selenomethionine in the roots (White 2016). The OsSULTR1.2, a sulfate transport, might regulate selenate uptake in rice (Liang et al 2019). On the other hand, selenite uptake by root cells can be catalyzed by OsNIP2.1, a silicon influx transporter (Zhao et al 2010). Moreover, the OsPT2, a $P_i$ transporter, enhance selenite uptake by rice roots (Zhang et al 2014a). Overall, the accumulation of Se in rice roots mediated by OsSULTR1.2, OsNIP2.1 and OsPT2 was accompanied by a slight increase in transport to shoots (Gupta et al 2017), because most of the absorbed selenite is metabolized to selenomethionine and tends to accumulates in the root (Wang et al 2012). Conversely, overexpression of NRT1.1B, a nitrate uptake transporter, in rice significantly improved selenomethionine translocation from roots to shoots, which was associated with increased accumulation of Se in grain when rice plants were fertilized with selenite (Zhang et al 2019). As nitrate positively regulates the expression of NRT1.1B in rice (Wang et al 2018), it is possible that the ability of NRT1.1B to regulate Se uptake in rice plants is dependent on the nitrogen availability during the growing season. However, information on how nitrogen availability affects Se uptake and accumulation in rice grains is hitherto lacking.

The increase in Se uptake is expected to have positive effect on rice grain yield as a result of increased number of tillers per plants, grains per panicle and percentage of filled grains (Wang et al 2013, Liu et al 2020). In addition to increasing yield, Se affects the concentration of minerals in rice grain (Boldrin et al 2013, Reis et al 2018). For example, selenite applied to the soil reduced B concentration in rice grains, while enhanced concentrations of Mg and Mn (Boldrin et al 2013). Moreover, Mo concentration in rice grains of the cultivar AN Cambará decreased with increasing Se supply (Reis et al 2020). Rice is good source of mineral elements that are important for human nutrition (Saleh eta al 2019). In this context, nitrogen fertilization has a direct impact on rice production and concentration of minerals in grain (Lin et al 2014, Wang et al 2018). Hao et al (2007) reported that nitrogen supply increased the concentrations of Cu, Fe, Mn and Zn in rice grains. On the other hand, nitrogen fertilization reduced the redistribution of N, Mg, Zn, K and Cu from bracts to grains, but increased the remobilization of P (Wang et al 2018). These results highlight the flexibility of nitrogen in the modulation of the mineral composition of rice grains. However, given that Se and nitrogen have the potential to modify the rice yield and grain nutritional quality, we asked how the combined effect of Se and nitrogen would affect the rice production and grain mineral composition.

In general, Se and nitrogen regulate primary metabolism in rice plants (Liang et al 2015, Malheiros et al 2019). The application of Se in rice increases yield and harvest index, which seems to be associated with increases on photosynthesis (Zhang et al 2014b). Rice grains are characterized by a high concentration of starch (Saleh eta al 2019). In this context, the negative effect of drought stress on starch accumulation in rice grains may be mitigated by Se treatment (Emam et al 2014). Moreover, Se increases concentrations of amino acids and proteins such as globulin, glutelin, prolamin and albumin in rice grains (Hu et al 2018, Reis et al 2020). Taken together, these studies suggest that a coordination between the source capacity and sink demand under Se treatment should lead to an improved grain nutritional quality. Nitrogen supply has a direct effect on A by affecting the levels of chlorophyll and photosynthetic proteins (Yin et al 2019). In addition, nitrogen might indirectly control A through alteration in the growth and accumulation of carbohydrates (Tschoep et al 2009). Despite many reports in the literature on the effect of Se and nitrogen on reproductive development of rice, little information is currently known about the combined effect Se and nitrogen in grain composition in terms of storage products such as starch, amino acid and protein. Thus, an understanding of relationship between the Se application and nitrogen fertilization would open up new perspectives to improve nutritional composition of rice grains.

Here we tested the hypothesis that Se uptake by roots is enhanced by nitrogen supply and thus regulates rice grain composition in term of storage products and important minerals. Furthermore, we examined the combined effect of Se and nitrogen supply on gas exchange and development of rice plants because vegetative and reproductive growth of rice varies with availability those minerals (Wang et al 2018, Malheiros et al 2019, Liu et al 2020).

## 2. Material and methods

### 2.1 Plant material and growth conditions

Seeds of rice (Oryza sativa L. ssp japonica cv Oochikara) were obtained from the plants grown in a greenhouse in Viçosa (20° 45'S, 42° 54'W, 650 m altitude), Minas Gerais, Brazil. Seed germination and early seedling growth were performed as described by Malheiros et al (2020). The plants were grown on a 1:1 (v/v) mixture of commercial substrate (Tropstrato HT, Mogi Mirim, Brasil) and soil in plastic pots with a 10 L capacity. The chemical characteristics of experimental soil are described in Table S1. The rice plants were maintained in a greenhouse with controlled air temperature (30/25 °C, day/night) and natural illumination. The maximum photosynthetically active radiation (PAR) was ~1000 μmol photons $m^{-2}$ $s^{-1}$ inside the greenhouse at midday. During the first week, the plants were watered daily to field capacity with deionized water, after which the plants were watered once a week with 500 ml of Hoagland's solution containing 0, 16 or 24 mM nitrogen. The characteristics of each nutrient solution are described in Table S2. Additionally, plants were divided into two groups: one received daily 500 ml of 10 μM sodium selenite solution (+Se plants) over the course of experiment, and in the other group the plants were watered daily with 500 ml deionized water (-Se plants). The positions of the pots were rotated twice a week, avoiding having the same treatment clustered together.

### 2.2 Determination of agronomic traits

The rice plants were harvested 140 days after planting and separated into stems, leaves, roots, panicles and grains with scissors. Total leaf areas were measured with an area meter (LI-3100, LICOR Inc, Lincoln, NE, USA). Plant tissues were then oven-dried at 60 °C until constant mass, and weighed to determine the dry masses of the vegetative and reproductive parts. At harvest, the following parameters were also measured: number of panicles and tillers, total grain yield, percentage of filled grain, 1000-grain mass and harvest index.

## 2.3 Photosynthetic measurements

Gas exchange was measured in flag leaves using an open-flow gas exchange system infrared gas analyzer (LI-6400XT, LICOR, Lincoln, NE, USA) fitted with a fluorimeter (LI-6400-40, LICOR, Lincoln, NE, USA). The analyses were performed under artificial PAR of 1000 μmol photons $m^{-2}$ $s^{-1}$ and the reference $CO_2$ concentration was maintained at 400 μmol $mol^{-1}$. During the measurements, the air flow into the chamber was 500 μmol air $s^{-1}$, while chamber temperature was kept at 25 $^{o}$C, with a leaf-to-air vapour pressure difference (VPD) of c. 1 kPa. For measurements of mitochondrial respiration rate in the dark ($R_d$), rice plants were kept in the dark at least 1 h, after which time dark respiration of the leaf was measured as described by Niinements et al (2006).

## 2.4 Mineral analysis

One hundred grains from each sample were selected at random from the seed yield samples and separated into husk and endosperm. Grains were oven-dried at 60 $^{o}$C until constant mass and stored in ziplock bags until analysis. The samples (0.5g) were digested with $HNO_3$:$HClO_4$ (10:1, v/v) and analyzed for concentration of P, K, Ca, Mg, S, Cu, Fe, Mn, Zn and Mo using an inductively coupled plasma-optical emission spectroscopy (ICP-OES, Perkin-Elmer, Shelton, CT) as described by Wheal et al (2011). The grain concentrations of N were determined by the Dumas combustion method (Muñoz-Huerta et al 2013). To quantify the concentration of Se, brown rice and root tissues were digested and quantified as described by El Mehdawi et al (2018).

## 2.5 Biochemical analysis

Biochemical analysis was performed on the same samples as used for mineral determination by ICP-OES. The procedure of extraction and assay of globulin, glutelin, prolamin, albumin and total protein was performed according to the method described by Reis et al (2020), with 100 mg of ground grains. The quantification of total chlorophyll and total carotenoids was performed as described earlier in Lichtenthaler (1987). Measurements of soluble sugar, starch and total amino acid were performed on 100 mg of ground grains according to Cross et al (2006).

### 2.6 Real-time quantitative PCR (RT-qPCR)

Extraction of total RNA from frozen ground root tissue and cDNA synthesis was performed as described by Malheiros et al (2020). RT-qPCR was performed with a mixture of cDNA, SYBR Green Master Mix and gene-specific primers (Table S3) according to Schippers et al (2008). Oligonucleotide sequences used for expression of OsNRT1;1B, OsSULTR1;2, OsPT2 and OsNIP2;1 were taken from Liang et al (2019) and Zhang et al (2019). The rice housekeeping gene ACTIN (Os03g50885) was used as an internal control to normalize the gene expression data: $\Delta C_T = C_T$ (gene) - $C_T$ (ACTIN), where $C_T$ stands for cycle threshold.

### 2.7 Statistical analysis

The statistical design was completely randomized distribution, using a 3x 2 factorial scheme. The experimental unit of the experiment consisted of four plants per plastic pot with four replicates per treatment. The data were subjected to analysis of variance ($P < 0.05$) using the SPSS (Statistical Package for the Social Sciences) 11.0 version, and significant differences between treatments were analyzed using the Tukey test at $P < 0.05$. The differences between plants treated and non-treated were analyzed by the Student’s t-test at the 5% level of significance.

## 3. Results

### 3.1 Similar changes in pigment composition and gas exchange occur in Se-treated plants with respect to plants fertilized with nitrogen

Given the evidence that low concentration of Se increases chlorophyll concentration in rice (He et al 2019), we hypothesized that Se may also function in regulating the content of chlorophyll related to nitrogen availability. The rice plants treated with Se did not display obvious alteration in the accumulation of chlorophyll compared with plants under nitrogen treatment alone (Fig. 1a). To confirm that Se did not induce alteration in the content of photosynthetic pigments in rice plants grown under different nitrogen fertilization regime, we determined the concentrations of chlorophyll and carotenoid in leaves of rice. The concentrations of total chlorophyll and total carotenoid were higher in plants grown in 16 mM and 24 mM nitrogen compared with nitrogen-limited plants (Fig. 1b, c). A similar trend was found in rice plants treated with Se when nitrogen supply was increased. Moreover, the net $CO_2$ assimilation rate (A) and stomatal conductance ($g_s$) showed a significant increase in rice plants grown in 16 mM and 24 mM nitrogen compared with nitrogen-limited plants (Fig. 1d, e). Rice plants treated with 24 mM nitrogen showed a significant increase in the transpiration rate (E), while 16 mM nitrogen did not affect the E compared with nitrogen-limited plants (Fig. 1f). There was no significant effect of Se on concentrations of pigments, A, $g_s$ and E in the three nitrogen regime. Intercellular-to-atmospheric $CO_2$ ration ($C_i/C_a$) was not significantly affected by Se and nitrogen supply (Fig. 1g).

### 3.2 Selenium supply modifies slightly the relationships between the availability of nitrogen and development in rice plants

Nitrogen supply increased leaf, culm and root biomass of rice plants (Fig. 2a-c). Consequently, the total biomass was also depended on the nitrogen supply (Fig. 2d). On the other hand, Se had no effect on leaf and culm biomass in most treatments, except at 24 mM nitrogen where a decrease in leaf biomass for Se was observed (Fig. 2a, b). Moreover, the root biomass was higher in Se plus16 mM nitrogen and lower in Se plus 24 mM nitrogen than in plants grown at a comparable nitrogen supply (Fig. 2c). There was a slight decrease in total biomass in plants treated with Se plus 24 mM nitrogen compared with plants at 24 mM nitrogen alone (Fig. 2d). The effect of Se on total leaf area depended on the nitrogen supply such that Se had no effect on leaf area at a low nitrogen supply, but Se led to a decrease of leaf area in plants growing at 16 mM nitrogen, and an increase of leaf area at 24 mM nitrogen (Fig. 2e). In addition, tiller number, panicle number, grain number and grain biomass increased with increasing nitrogen supply, independent of the Se treatment (Fig. 2f-i). The 1000-grain weight was slightly decrease in rice plants treated with 16 mM nitrogen, but not in plants grown at 24 mM nitrogen compared to nitrogen-limited plants (Fig. 2j). Treatment with Se had no effect on 1000-grain weight in plants with a limiting nitrogen supply, whereas Se increased slightly 1000-grain weight in the high nitrogen treatments (Fig. 2j). No differences between treatments were found in the filled grain percentage and harvest index (Fig. 2k, l).

### 3.3 Selenium uptake and allocation to the grain is altered due to changes in the soil nitrogen availability

Given the evidence that the expression level of OsNRT1.1B, OsPT2, OsSULTR1.2 and OsNIP2.1 in root affects Se uptake and transport in rice plants (Liang et al 2019, Zhang et al 2019), we asked whether Se and nitrogen modulate expression of those genes. The expression of OsNRT1.1B increased by 4,0 and 5,6-fold in root of rice plants grown at 16 mM and 24 mM nitrogen, respectively (Fig. 3a). Moreover, the effect of increased nitrogen concentration on the expression of OsNRT1.1B did not depend of Se supplied, since Se and nitrogen together produced the same OsNRT1.1B expression in root as nitrogen alone. A significant increase in the abundance of OsSULTR1.2 and OsPT2 transcripts was observed in roots of plants grown in 16 mM and 24 mM nitrogen compared with nitrogen-limited plants (Fig. 3b, c). Se treatment combined with nitrogen treatment increased the expression of OsSULTR1.2 and OsPT2 more than nitrogen supplies alone. Expression of OsNIP2.1 was upregulated in root of plants treated with Se at all three nitrogen supplies (Fig. 3d). In contrast with Se treatment, we did not observe any significant changes in expression of OsNIP2.1 with increasing nitrogen concentrations. We examined the effect of changing transcript abundance on Se concentration in both root and grain. There was a slight increase in Se concentration in both root and grain of plants treated with 16 mM and 24 mM nitrogen compared with nitrogen-limited plants (Fig. 3e). On the other hand, treatment with Se led to a progressive increase of Se concentration in both root and grain as the nitrogen supply was increased.

### 3.4 Selenium affects allocation of mineral elements to the seeds in response to soil nitrogen availability

A significant increase in concentrations of N, P, S, Ca and Fe was observed in grain of rice plants treated with Se grown in a nitrogen-limiting conditions (Fig. 4a-e). There was also an increase of N, P, S, Ca and Fe in grain as the nitrogen supply increased. However, N concentration was higher in grain of plants treated with Se plus 24 mM nitrogen than in grain of plants grown in 24 mM nitrogen alone (Fig. 4a). Moreover, Se did not alter the concentration of P and S in grain of rice plants grown in 16 or 24 mM nitrogen supply (Fig. 4b, c). There was an increased Ca concentration in grain of plants treated with Se, independent of the concentration of nitrogen supplied (Fig. 4d). Our results also revealed that Fe concentration increased in grain of plants treated with Se plus 24 mM nitrogen compared to nitrogen alone (Fig. 4e). Interestingly, Fe concentration in grain of plants under Se treatment was higher in nitrogen-limited plants than in plants grown in 16 or 24 mM nitrogen. The concentration of Mn in grain was reduced by nitrogen supply, and this effect was not influenced by Se (Fig. 4f). On the other hand, Se tended to reduce concentrations of Cu and Zn in grain of plants treated with increasing nitrogen supply (Fig. 4g, h). In contrast with effect of Se in concentration of Cu and Zn, Se increased concentration of Mg and Mo in grain of plants grown in 16 mM and 24 mM nitrogen compared with nitrogen-limited plants (Fig. 4i, j). It is interesting to note that no statistical differences were found in concentrations of Cu, Zn, Mg and Mo in grain between plants treated with nitrogen and Se plus nitrogen within the same nitrogen supply (Fig. 4f-j). There were no differences in concentrations of K and B in grain across treatments (Fig. 4k, l).

### 3.5 Effects of Se and soil nitrogen availability on rice grain nutritional composition

Overall, concentrations of albumin and globulin in rice grain were not affected by nitrogen supply (Fig. 5a, b). However, albumin and globulin in grain of plants treated with Se plus 24 mM nitrogen was higher than in grain of plants treated with Se in nitrogen-limited conditions. There was a significant increase in prolamin concentration in grain of plants treated with 24 mM nitrogen, but not in plants grown in 16 mM nitrogen, when compared with plants in nitrogen-limited conditions (Fig. 5c). We did not observe any significant effect of Se in grain prolamin concentration with increasing nitrogen concentration (Fig. 5c). However, our results also revealed that Se increased prolamin concentration by 1.44, 1.46 and 1.31-fold in plants grown in 0 mM, 16 mM and 24 mM nitrogen, respectively, as compared with plants grown at a comparable nitrogen supply. There was a significant increase in glutelin concentration in grain of plants treated with 16 mM and 24 mM nitrogen compared with nitrogen-limited plants (Fig. 5d). Treatment with Se increased concentration of glutelin in grain of plants grown in 16 mM and 24 mM nitrogen compared with nitrogen-limited plants. Additionally, glutelin concentration was higher in grain of plants treated with Se plus 16 mM nitrogen and Se plus 24 mM nitrogen than in grain of plants grown in 16 mM and 24 mM nitrogen alone (Fig. 5d). Total protein increased in grain of plants treated with16 mM and 24 mM nitrogen compared with nitrogen-limited plants (Fig. 5e). Moreover, Se led to an increase of total protein in grain of plants grown in 16 and 24 mM nitrogen compared with nitrogen deficient plants. The results also showed an increased concentration of total protein of 16.7% and 21.0% in grain of plants treated with Se plus 16 mM nitrogen and Se plus 24 mM nitrogen, respectively, as compared with plants grown at a comparable nitrogen supply (Fig. 5e). A significant increase in concentration of total amino acids was observed in grain of plants grown in 24 mM nitrogen compared with nitrogen-limited plants (Fig. 5f). Se led to a significant 32.2% and 95.2% increase of total amino acid in grain of plants grown in nitrogen limited condition and in 16 mM nitrogen, respectively, when compared with plants grown at a comparable nitrogen supply (Fig. 5f). The concentration of soluble sugar increased in grain of rice plants treated with 24 mM nitrogen, but not in plants grown at 16 mM nitrogen compared to nitrogen-limited plants (Fig. 5g). Similar results were obtained in studies comparing the effects of Se at three nitrogen supplies in concentration of soluble sugar. Moreover, the effect of increased nitrogen concentration on the concentrations of soluble sugar and starch did not depend of Se supplied, since Se and nitrogen together produced the same concentrations of these metabolites in grain as nitrogen alone (Fig. 5g, h).

## 4. Discussion

In rice, overexpression of NRT1.1B, a gene encoding a nitrate uptake transporter, increases Se uptake and transport, which in turn increases Se concentration in the grains (Zhang et al 2019). However, the coordination of Se and NRT1.1B action by nitrogen remains hitherto unclear, particularly during reproductive development in rice. The results of the present study showed that Se did not affect the relationships between nitrogen and reproductive development in rice plants. However, Se increased the concentration of a range of minerals in grain in response to nitrogen treatments.

As expected (Duan et al 2007, Che et al 2015), total leaf area, biomass production and grain yield reduced in rice plants that receive a suboptimal nitrogen supply (Fig. 2). However, looking at total leaf area, biomass production and grain yield as a function of Se within the nitrogen treatments suggested that Se has no effect on yield components of rice plants. This is somewhat at odds with earlier reports showing that Se increases photosynthetic performance and grain yield in rice (Wang et al 2013, Zhang et al 2014, Deng et al 2017). Several factors could explain this discrepancy, including the fact that we are studying different cultivar subject to different nutrition. Irrespective of the reason underlying the different conclusions of these studies, the observed results would be consistent with the notion that Se does not change the coordination between the source capacity and sink demand as nitrogen supply is increased. In our experimental setup, nitrogen caused an increase in expression of OsNRT1.1B in root (Fig. 2), which was shown to lead to higher Se transport to the grains from the roots (Liang et al 2019, Zhang et al 2019). These results suggest a role for OsNRT1.1B in nitrogen-dependent increases in Se uptake and transport to the grains. In fact, concentration of Se increased in both root and grain by Se treatment in a nitrogen-dependent manner (Fig. 3). Moreover, the ability of Se to strongly increase the effect of nitrogen supply in expression of OsSULTR1.2, OsPT2 and OsNIP2.1 in root, suggests that Se uptake mediated by influx carrier expression are necessary for the full response to nitrogen. Consistent with this view, Se concentration in root was positively associated with Se treatment combined with nitrogen supply, mirroring the Se accumulation patterns in the grain. Together, these findings indicate that nitrogen is necessary for the response to Se accumulation in rice grain, in which stimulation of Se uptake and transport are linked.

Rice grains are good source of minerals for human nutrition (Saleh et al 2019). In this context, Se has been proposed to affect mineral composition of rice grain (Boldrin et al 2013, Emam et al 2014). Our study indicated that Se itself significantly increased the concentrations of N, P, Ca, S and Fe as compared to plants that receive a suboptimal nitrogen supply (Fig. 4). Mineral concentrations may be reduced in the grains when the number of grain produced is increased and conversely increased in concentration when the number of grains produced in decreased (Murphy et al 2008). Comparison of the Se and limiting nitrogen supply responses suggest that enrichment of seed mineral concentration is not a direct function of reduced grain yield per plant or grain biomass since grain yield of plants treated with Se alone is similar to that observed in the nitrogen-limited plants (Fig. 2). Interestingly, concentrations of N, P, Ca, S and Fe showed an increase in nitrogen-replete plants compared to nitrogen-limited plants, a result that was associated with increase in grain yield (Fig. 4). These results imply that dilution by increased grain yield at nitrogen-fertilized plants may not be a factor affecting mineral concentration in rice grain. In addition, Se application to nitrogen-fertilized plants (16 mM and 24 mM nitrogen) did not affect the concentration of P and S whereas concentrations of N, Fe and Ca was increased compared with plants grown upon 16 mM or 24 mM nitrogen (Fig. 4). Together, these findings suggest that the observed responses are coordinated by a complex interaction involved plant and soil responses to the treatments. However, molecular evidence about the regulatory mechanisms under Se and nitrogen supply remains to be defined.

Treatment of rice plants with Se causes an increase in concentration of Cu, Zn and Mn in grains (Boldrin et al 2013). In general, our results revealed that Se plus nitrogen supply led to a decrease in Cu, Zn and Mn in rice grains (Fig. 4). Interestingly, Se also affected the concentrations of N, P, Ca, S, Fe and Mg in rice grain (Fig. 4). Thus, Se and nitrogen affected minerals that move by mass flow such as Ca, Mg and S, and also those with diffusion to the root as the main mechanism, including P, Mn, Zn, Cu and Fe (White and Greenwood 2013). The amount of minerals in seed is the result of the balance between uptakes into the root, distribution and partition to the seeds (Yoneyama et al 2016). Se negatively regulates the lateral root development in rice (Malheiros et al 2019), which presumably could reduce uptake of nutrients, contributing to the change in grain mineral concentration. In this sense, there was no connection between the grain mineral concentration and the rate of root growth in rice plants treated with Se and nitrogen (Figs 2 and 4). Together, these findings suggest that the effect of

Se and nitrogen on grain minerals does not associate with the mode of mineral delivery and uptake into the rice plants.

Concentrations of albumin and globulin are not limited by nitrogen supplies (Fig. 5). The content of these proteins is most influenced by the genotype than the supply of nitrogen (Ning et al 2010). Moreover, a positive effect was found in grain of plants treated with Se plus 24 mM nitrogen compared with plants exposed to Se in nitrogen-limited conditions. It is also noteworthy that the prolamin concentration showed higher accumulation in grain of plants treated with Se combined with nitrogen than in grain of plants grown in nitrogen supplies alone. Moreover, Se was able to maintain prolamin accumulation even under low nitrogen conditions. The impact of Se on the accumulation of grain glutelin concentration depended on the nitrogen fertilization regime. In this context, the fact that glutelin accumulate considerably in grain of plants under Se treatment is interestingly because it suggests that control of glutelin biosynthesis could be mediated by the combined effect of Se and nitrogen supply. Storage proteins are the main sites of Se and nitrogen accumulation in rice. Given that glutelin accounted for as much as 80% of the total grain storage protein (Kawakatsu et al 2010), Se increased the total storage protein concentration when plants were growing with an optimal nitrogen supply, but not in nitrogen-limited plants. This result indicates a beneficial effect of Se on nitrogen metabolism, inducing a nitrogen redistribution to improve protein quality in rice grain, since this protein has higher lysine content and better digestibility in humans (Chen et al 2018). When nitrogen-replete plants are exposed to Se, the stimulation of photosynthesis is maintained, which will maintain export to the growing sinks. Consistent with this finding, starch and soluble sugars showed a similar trend in grain of plants non-treated and treated with Se, suggesting that Se did not changes the regulation of sugar and starch metabolism in rice grain.

## 5. Concluding remarks

Interactions between the Se and nitrogen does not increase the rice yield, but Se treatment combined with nitrogen treatment increase the expression of OsSULTR1.2 and OsPT2 in root more than nitrogen supplies alone, while the increase in expression of OsNIP2.1 is regardless of nitrogen supply in Se-treated plants. Concomitant to this, the supply of nitrogen, increase the relative expression of OsNRT1.1B, increase the translocation of Se from the root to the shoot of plants supplemented with Se, improving the content of Se in rice grains. Moreover, Se promote a significant increase in concentrations of N, P, S and Fe in grain of rice plants treated with Se grown in nitrogen-limiting conditions while the Ca concentration increase regardless of nitrogen supply. Besides, Se increase concentrations of Mg and Mo in grain compared with nitrogen-limited plants. Furthermore, the combination of Se plus nitrogen concentration promote a positive effect on caloric levels of grain, but a negative effect on the concentration of a range of minerals such Fe, Zn, Cu and Mn.

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# Figures

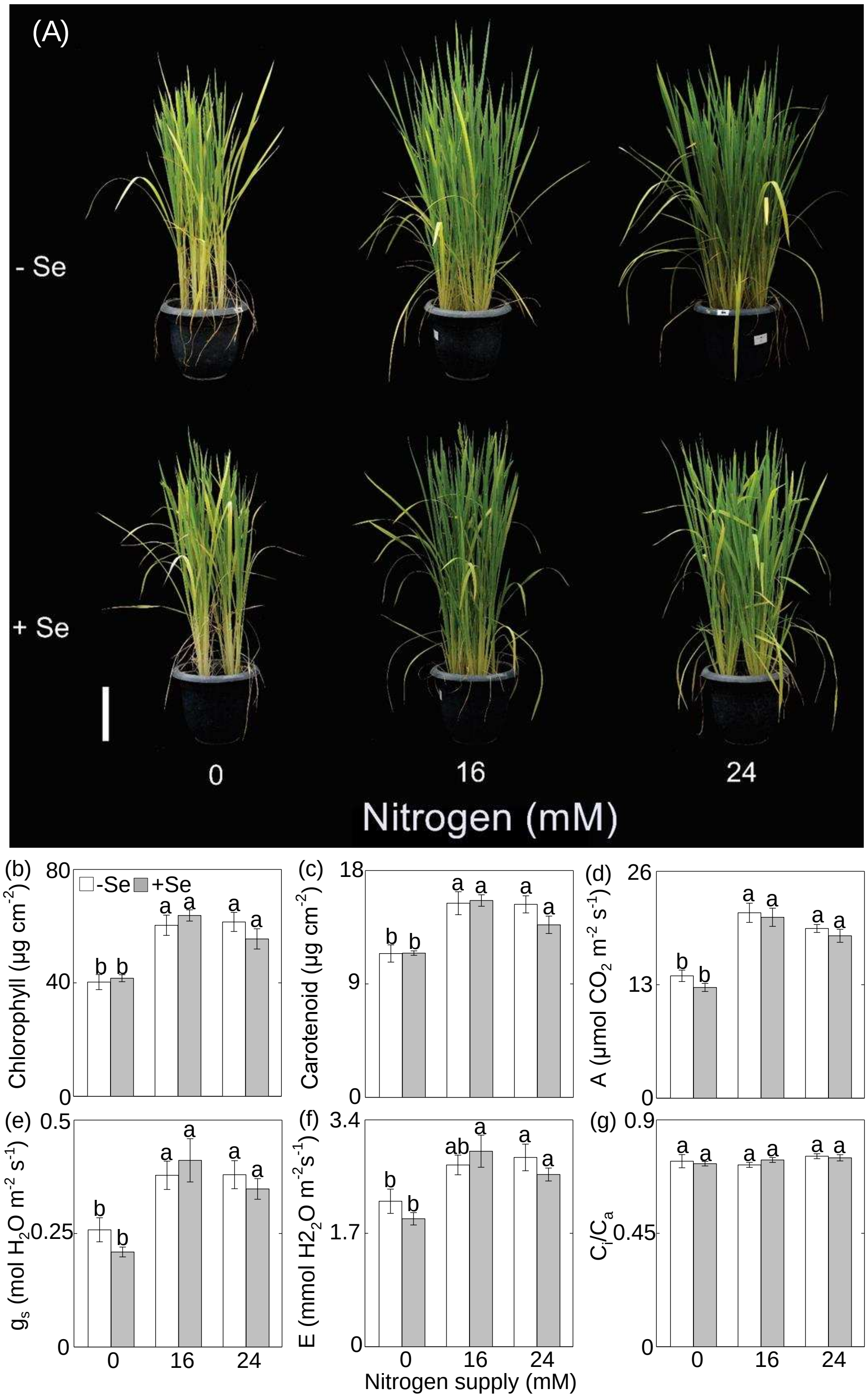

**Fig. 1** Phenotypic changes and physiological parameters observed in rice plants treated with Se and nitrogen, singly and in combination. (a) Phenotype of rice plants grown for 90 days under Se and nitrogen treatments. The scale bar represents 20 cm. (b) Total Chlorophyll concentration. (c) Total carotenoid concentration. (d) Net $CO_2$ assimilation rate. (e) Intercellular-to-atmospheric $CO_2$ ratio. (f) Stomatal conductance. (g) Transpiration rate. Bars labeled with the different letters indicate significant differences by Tukey's test at 5% probability. Data are mean ± standard error of three independent experiments, with four replicates each.

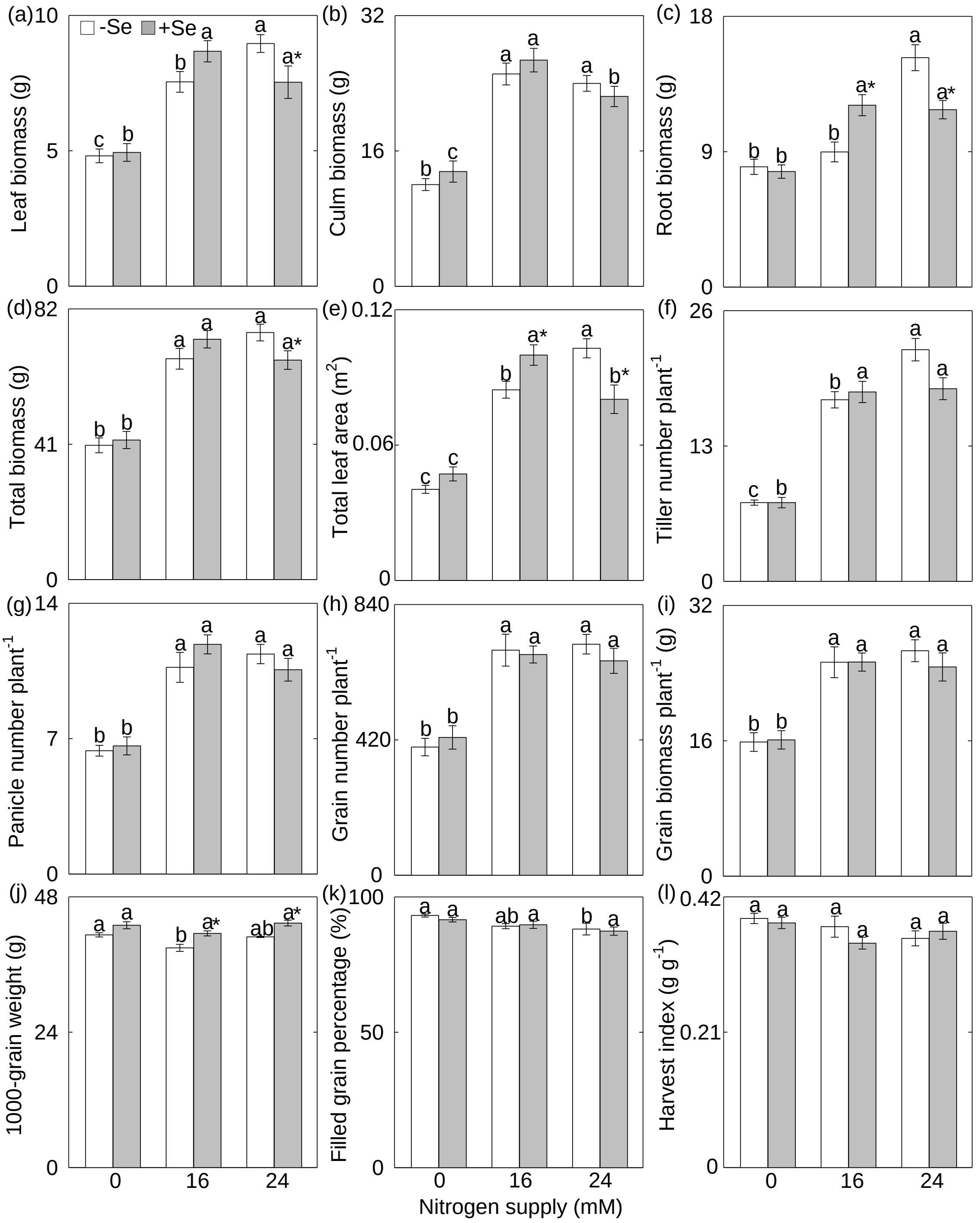

**Fig. 2** Effects of Se and nitrogen treatments, singly and in combination, on the yield components of rice plants. (a) Leaf biomass. (b) Culm biomass. (c) Root biomass. (d) Total biomass. (e) Total leaf area. (f) Tiller number. (g) Panicle number per plant. (h) Grain number per plant. (i) Grain biomass. (j) 1000-grain weight. (k) Filled grain percentage. (i) Harvest index. Bars labeled with the different letters indicate significant differences by Tukey's test at 5% probability. Asterisks indicate values determined by the Student's t-test to be significantly different between plants treated and non-treated with Se within each nitrogen application ($P < 0.05$). Data are mean ± standard error of three independent experiments, with four replicates each.

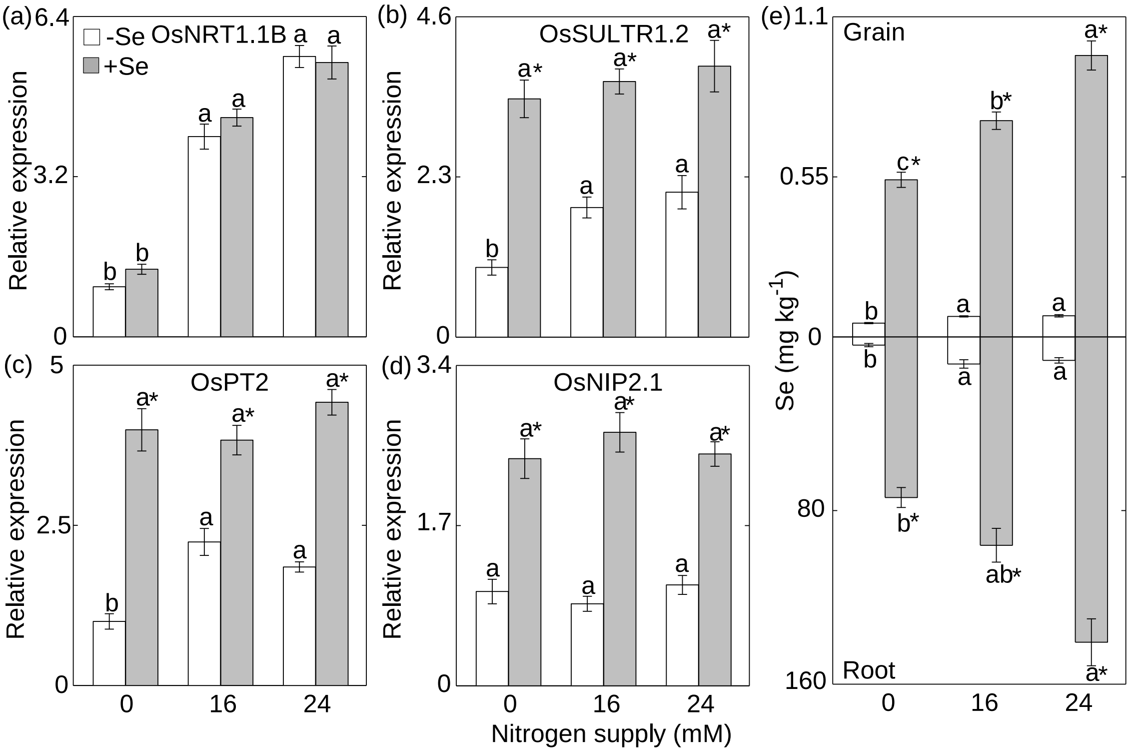

**Fig. 3** Impact of Se and nitrogen treatments, singly and in combination, on root gene expression, and Se content. (a-d) Relative expression level of OsNRT1.1B, OsSULTR1.2, OsPT2 and OsNIP2.1 in root. (e) Concentrations of Se in both root and grain. Bars labeled with the different letters indicate significant differences by Tukey's test at 5% probability. Asterisks indicate values determined by the Student's t-test to be significantly different between plants treated and non-treated with Se within each nitrogen application ($P < 0.05$). Data are mean ± standard error of three independent experiments, with four replicates each.

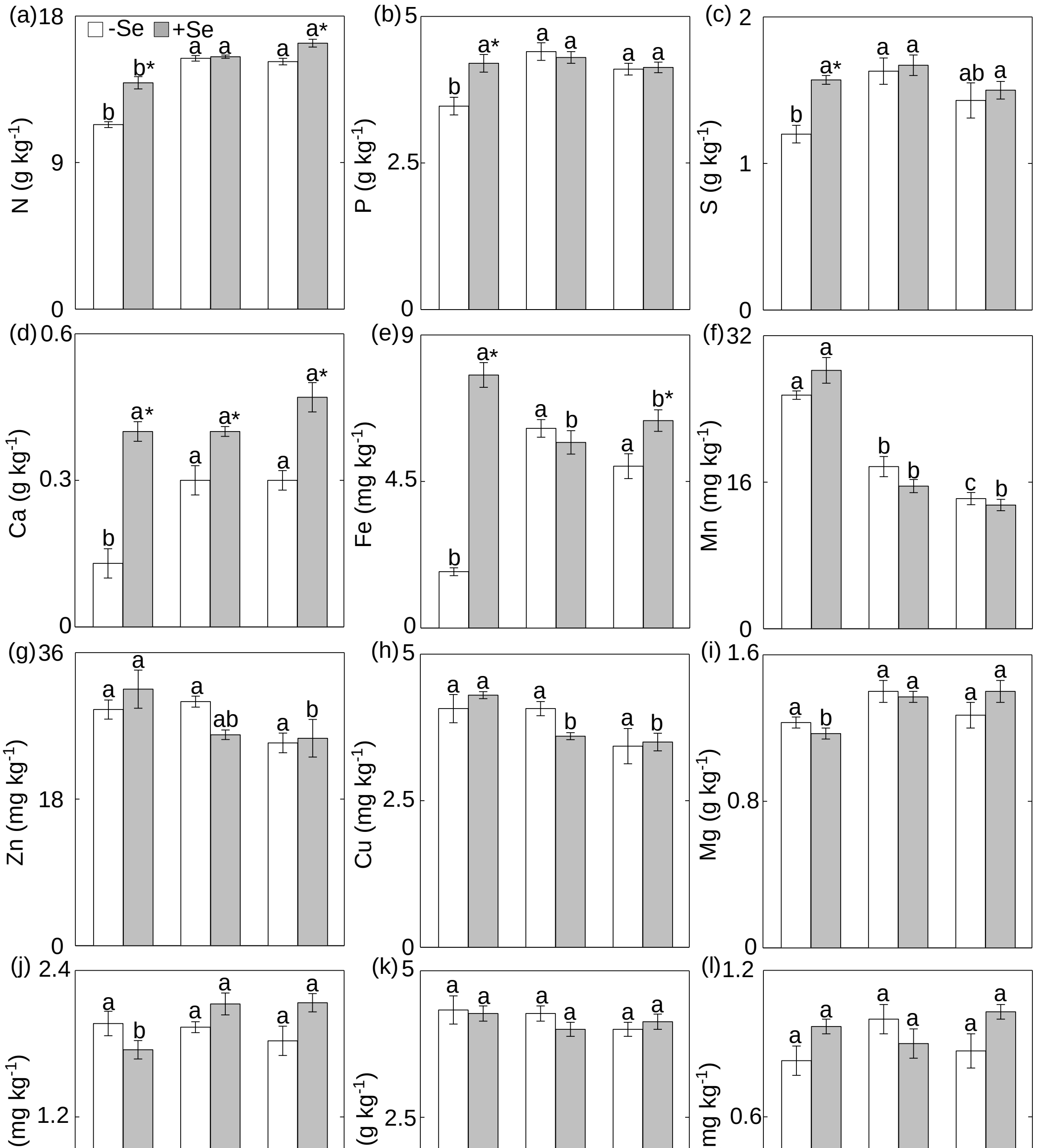

**Fig. 4** Impact of Se and nitrogen treatments, singly and in combination, on grain mineral composition. (a) Total nitrogen. (b) Phosphorus. (c) Sulphur. (d) Calcium. (e) Iron. (f) Manganese. (g) Zinc. (h) Copper. (i) Magnesium. (j) Molybdenum. (k) Potassium. (l) Boron. Bars labeled with the different letters indicate significant differences by Tukey's test at 5% probability. Asterisks indicate values determined by the Student's t-test to be significantly different between plants treated and non-treated with Se within each nitrogen application ($P < 0.05$). Data are mean ± standard error of three independent experiments, with four replicates each.

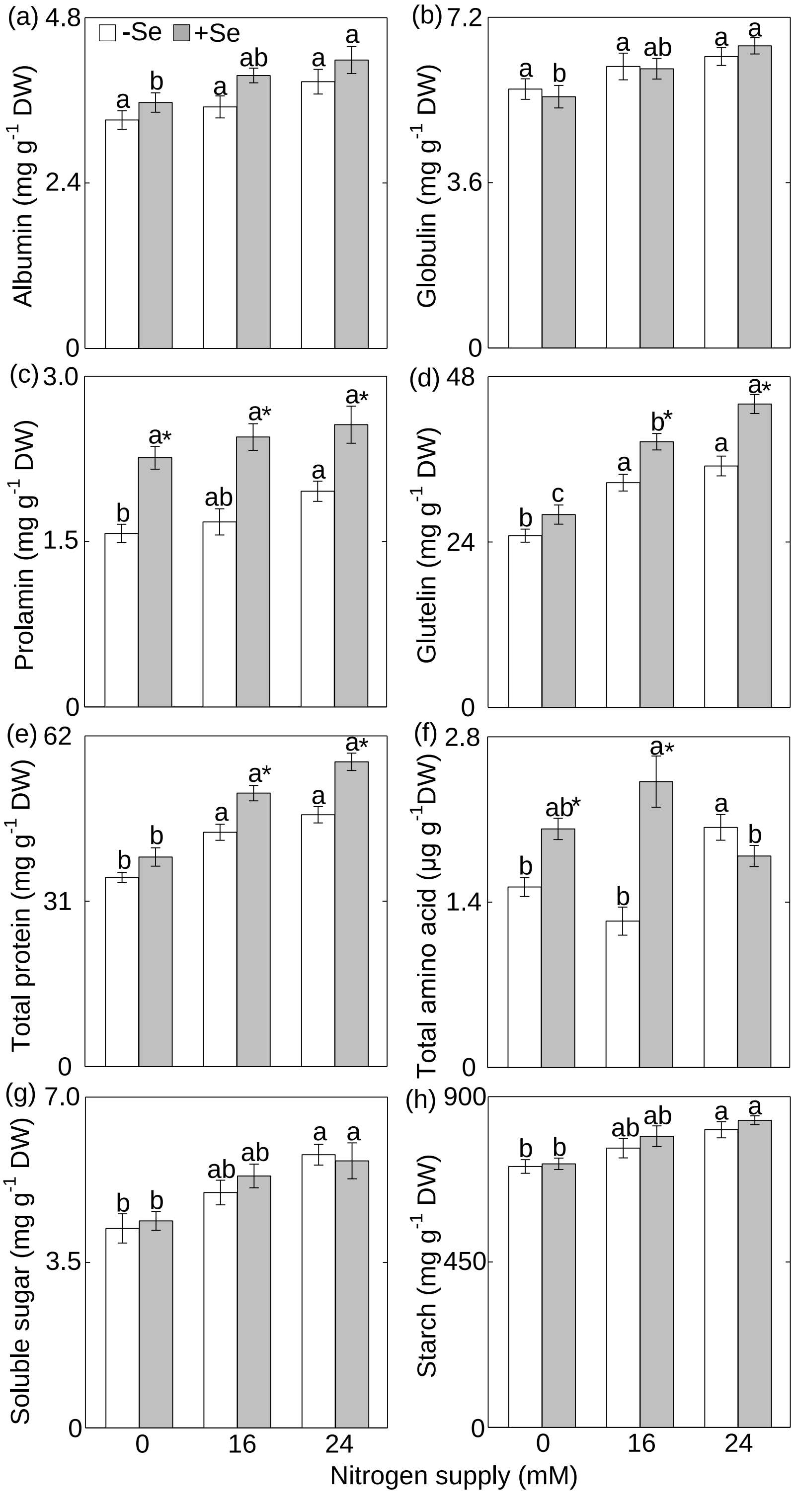

**Fig. 5** Changes in the concentrations of protein, amino acid, sugar and starch in grain of rice plants treated with Se and nitrogen, singly and in combination. (a) Albumin. (b) Globulin. (c) Prolamin. (d) Glutelin. (e) Total protein. (f) Total amino acid. (g) Soluble sugar. (h) Starch. Bars labeled with the different letters indicate significant differences by Tukey's test at 5% probability. Asterisks indicate values determined by the Student's t-test to be significantly different between plants treated and non-treated with Se within each nitrogen application ($P < 0.05$). Data are mean ± standard error of three independent experiments, with four replicates each.

## Supplementary material

**Suppl. Table S1.** Chemical properties of the soil employed in experiments. Data are means ± standard error of three replicates.

| Properties | Soil |
|---|---|
| pH | 6.3±0.04 |
| N (g $dm^{-3}$) | 1.4±0.01 |
| P (mg $dm^{-3}$) | 236.4±13.1 |
| K (mg $dm^{-3}$) | 690±20.2 |
| Ca ($cmol_c$ $dm^{-3}$) | 7.8±0.5 |
| Mg ($cmol_c$ $dm^{-3}$) | 3.8±0.2 |
| Al ($cmol_c$ $dm^{-3}$) | 0.0 |
| H+Al ($cmol_c$ $dm^{-3}$) | 3.6±0.1 |
| CEC ($cmol_c$ $dm^{-3}$) | 17.0±1.1 |
| m (%) | 0.0 |
| V (%) | 78.7±2.6 |

P, phosphorus; K, potassium; Ca, calcium; Mg, magnesium; Al, exchangeable aluminium; H+Al, exchangeable; acidities; CEC, cation exchange capacity; m, aluminium saturation and V, base saturation.

**Suppl. Table S2.** Composition of macronutrients in the Hoagland nutrient solution (Epstein 1972), with modifications, in order to provide three levels of Nitrogen. The composition of other micronutrients was maintained according to Epstein (1972).

| Salt | Stock solution | 0mM | 16mM | 24mM |
|---|---|---|---|---|
| KCl | 2 M | 2 mL/L | - | - |
| $KH_2PO_4$ | 1 M | 2 mL/L | - | - |
| $CaCl_2$ | 2 M | 2,5 mL/L | - | - |
| $CaCl_2$ | 0,5 M | - | 2 mL/L | - |
| $MgSO_4$ | 1 M | 2 mL/L | 2 mL/L | 2 mL/L |
| $KNO_3$ | 1 M | - | 6 mL/L | 6 mL/L |
| $NH_4H_2PO_4$ | 0,5 M | - | 4 mL/L | 4 mL/L |
| $Ca(NO_3)_2$ | 2 M | - | 2 mL/L | 2,5 mL/L |
| $NaNO_3$ | 2,5 M | - | - | 2 mL/L |
| $NH_4Cl$ | 0,2 M | - | - | 5 mL/L |

**Suppl. Table S3**. Sequences of oligonucleotides used for qRT-PCR.

| Locus | Name | Oligo | Sequence 5’→3’ |
|---|---|---|---|
| Os03g05640 | PT2 | PT2-F | CACAAACTTCCTCGGTATGCT |
| | | PT2-R | GAAACCCCACAAATCCACAAC |
| Os02g51110 | NIP2.1 | NIP2.1-F | GGGGCAATTTCAGGTGGATCG |
| | | NIP2.1-R | TTCTGGGAGGAGCCTTCCTT |
| Os10g40600 | NRT1.1B | NRT1.1B-F | GGCAGGCTCGACTACTTCTA |
| | | NRT1.1B-R | AGGCGCTTCTCCTTGTAGAC |
| Os03g0195500 | SULTR1.2 | Sultr1.2-F | TCAAAGAAGAACCCGCTAGATT |
| | | Sultr1.2-R | GCAATTCCAAGGAAGCCTTTAA |
| Os03g0718100 | ACTIN1 | Actin1-F | TCCATCTTGGCATCTCTCAG |
| | | Actin1-R | GTACCCGCATCAGGCATCTG |