Nitrogen

MDPI

Article

Cytokinin Biosynthesis Is Affected by Selenium and Nitrate Availabilities to Regulate Shoot and Root Growth in Rice Seedlings

Lubia S. Teixeira, Thiago A. L. Mota, Deisy J. C. Lopez, Victor A. Amorim, Carla S. Almeida, Genaina A. Souza and Dimas M. Ribeiro *

Department of Plant Biology, Federal University of Viçosa, Vicosa 36570-900, Brazil; lubia.silva@ufv.br (L.S.T.); thiago.lacerda@ufv.br (T.A.L.M.); deisy.j.lopez@ufv.br (D.J.C.L.); victor.amorim1@ufv.br (V.A.A.); carla.d.almeida@ufv.br (C.S.A.); genaina.souza@ufv.br (G.A.S.)
* Correspondence: dimas.ribeiro@ufv.br; Tel.: +55-31-3612-5162

Abstract: Selenium (Se) and nitrate have the potential to modify rice root architecture, but it is unclear how Se is linked to changes in the rice seedlings nitrate status. Thus, rice seedlings were grown in nutrient solutions containing either 0- or 10-µM Se that were supplemented with 0.05 (low nitrate condition) or 5.0 mM nitrate (high nitrate condition). Se application to seedlings treated with low nitrate led to sugar accumulation in shoot and root and increased cytokinin concentrations in root, while decreasing cytokinin concentrations in shoot compared with seedlings in 0.05 mM nitrate alone. This, in turn, resulted in decreased shoot growth, while downregulation of *OsXTH* and *OsEXP* negatively affected root expansion. On the other hand, Se combined with 5.0 mM nitrate did not affect sugar concentration in tissues compared with seedlings in 5.0 mM nitrate. Moreover, Se negatively regulated the cytokinin biosynthesis in shoot and root of seedlings grown under 5.0 mM nitrate. The reduction in cytokinin concentrations by Se under high nitrate condition decreased shoot growth, but increased root growth through induction of *OsXTH* and *OsEXP*. Thus, many of the effects of Se in shoot and root growth are due to a shift in nitrate status of the seedlings.

Keywords: cell expansion; *Oryza sativa*; shoot-root allocation; sodium selenite

Citation: Teixeira, L.S.; Mota, T.A.L.; Lopez, D.J.C.; Amorim, V.A.; Almeida, C.S.; Souza, G.A.; Ribeiro, D.M. Cytokinin Biosynthesis Is Affected by Selenium and Nitrate Availabilities to Regulate Shoot and Root Growth in Rice Seedlings. *Nitrogen* **2024**, *5*, 191–201. https://doi.org/10.3390/nitrogen5010013

Academic Editor: Marouane Baslam

Received: 15 January 2024
Revised: 22 February 2024
Accepted: 23 February 2024
Published: 4 March 2024

1. Introduction

Nitrogen is the most important nutrient for plant growth and development, with nitrate being usually the main source of inorganic nitrogen and ammonium also making contributions [1]. In this context, nitrate at low concentration can stimulate rice seminal root growth by decreasing cytokinin biosynthesis [2]. As a result, reduction in cytokinin concentrations act as a positive regulator of the expression of *expansin* (*EXP*) and *xyloglucan endotransglucosylase/hydrolase* (*XTH*) genes resulting in expansion of seminal root. On the other hand, the increasing in nitrate supply increases expression of *isopentenyl transferase* (*IPT*) and *cytochrome P450 monooxygenase* (*CYP735A*) genes, resulting in accumulation of cytokinin and inhibition of primary root elongation of rice [3,4]. Taken together, these studies suggest that the nitrate is effective in modulating root system growth by modifying cell-wall dynamics. Interestingly, the accumulation of cytokinin in root mediated by nitrate supply was accompanied by an increase in transport to shoot via ABCG14 transporter [5]. Thus, it is possible that the relative rate of shoot and root growth is modulated by cytokinin signals related to the nitrate status of the seedlings.

Seedling growth strategy is one of the key factors determining the survival and population succession of plants in their habitats [6]. An adaptive strategy for seedlings is altering root morphology to improve soil exploration for nitrogen [7]. In this context, the growth response of seedlings to different nitrogen levels involves modification in biomass allocation patterns. There is a positive correlation between nitrate supply and shoot-root

allocation in tobacco plants [8]. In this sense, high nitrogen fertilization reduces the rate of root growth compared with shoot growth, while nitrogen-deficient plants tend to reduce shoot growth and stimulate root growth, so that the plants can presumably improve mineral uptake from soils [9,10]. However, it is so far unclear how nitrogen-dependent response for cytokinin accumulation acts to alter partitioning of biomass of rice seedlings to maintain a functional equilibrium between shoot and root growth in response to nitrate supply.

The application of selenium (Se) alone also modifies root system architecture of rice seedlings through changes in biosynthesis of auxin and ethylene [11]. In addition, it has been demonstrated that Se may modify the carbon and nitrogen metabolism in root and shoot [12]. As Se positively regulates the accumulation of sugars in plant tissues [13], it is possible that the ability of nitrogen to regulate cytokinin biosynthesis in plants is dependent on the carbon availability. Rice roots obtain Se in the form of selenite, mainly in floodplain soils where irrigated rice is produced [14,15]. Molecular and physiological analysis in *Arabidopsis* has demonstrated that treatment with 10 μM and 40 μM selenite increases the in situ expression of cytokinin-inducible primary response gene (*ARR5*), which seems associated with root growth inhibition [16]. In fact, the expression of *EXP* genes is regulated by selenite in roots of rice seedlings [11]. These studies suggest that a balance between nitrogen and cytokinin plays a significant role during plant growth and that Se could in some way affect the nitrogen-cytokinin balance. Despite many studies reporting the effect of nitrate and Se in isolation on plant growth [2,5,12,16], relatively little is known concerning the effect of Se in controlling rice seedling growth following exposure to nitrate. An open question therefore is how Se and nitrate conditions may interact to affect shoot and root growth of rice seedlings. Thus, the elucidation of the mechanisms that control rice seedling growth in response to Se application and nitrate fertilization are needed to assess the consequences for seedling performance during the vegetative growth stage.

In this study, we test the hypothesis that Se induces changes in the interaction between nitrate supply and cytokinin biosynthesis to mediate control of shoot and root growth of rice seedlings. In addition, we investigated the ability of Se to alter sugar biosynthesis and thus regulates cytokinin biosynthesis and rice seedling growth in response to nitrate availability.

2. Materials and Methods

2.1. Growing Conditions and Experimental Design

Seeds of rice (*Oryza sativa* L. ssp. *japonica* cv 'Oochikara') were surface-sterilized, soaked, and germinated as described by Malheiros et al. [17]. Upon germination, seedlings with a 2-cm-long radicle were transferred to 1.5 L pots (20 seedlings per pot) filled with Hoagland and Arnon [18] nutrient solutions containing either 0- or 10-μM Se that were supplemented with 0.05 (low nitrate condition) or 5.0 mM nitrate (high nitrate condition). In addition, seedlings were grown in the Hoagland and Arnon solution supplemented with 10 μM sodium selenite. The pH was adjusted daily to 5.5 and the solution was renewed every 2 days. The hydroponic experiments were carried out in a growth chamber (Forma Scientific Inc., Marietta, OH, USA) under 16/8 h day/night cycle (30/24 °C) with 60/75% relative humidity and 150 μmol m^{-2} s^{-1} light intensity. After 7-days growth, seedlings were harvested, thoroughly rinsed in deionized water, and then used for the following experiments. Values plotted are the mean of three separate experiments, with five replicates for phenotypic assays, concentrations of cytokinin, sugars, nitrate, and selenium, and three replicates for gene expression.

2.2. Vegetative Growth Assessment

At each harvest, the shoot and root were separated using a scalpel and then shoot and root length were performed using a Vernier caliper. Rice roots were transferred immediately to 30% (*v*/*v*) ethanol to avoid any desiccation, and then the root systems were scanned using a desktop scanner and analyzed using the image-processing software WinRhizo pro V 2008b (Regent Instruments Inc., Quebec, QC, Canada) as described by Zhu et al. [19].

The shoots and roots were subsequently put in paper bags and oven-dried at 60 °C until constant mass to determine dry mass.

2.3. Measurements of Nitrate, Selenium, and Sugars

For Se analysis in the rice seedlings, shoot and root dried at 60 °C until constant mass were digested in nitric acid and the inductively coupled plasma optical analysis were performed as described previously in El Mehdawi et al. [20].

The analysis of total sugar and nitrate was performed using 100 mg of freeze-dried samples of shoot and root. Sugars and nitrate in shoot and root tissues of rice seedlings were extracted and quantified as described by Cross et al. [21]. Assays were performed in 96-well microplates, and sugar and nitrate absorbances were measured at 340 nm and 540 nm, respectively, using an Elisa reader (Tunable Microplate Reader, VersaMax, Sunny Vale, CA, USA).

2.4. Cytokinin Analysis

Extraction and determination of cytokinin from samples of 0.3 g shoot and root of rice seedlings by high-performance liquid chromatography (HPLC) were performed according to the method described by Song et al. [22]. Standard cytokinin fraction samples [zeatin, zeatin riboside, and N^6-(Δ^2-isopentenyl) adenine] were obtained from Sigma-Aldrich (St. Louis, MO, USA).

2.5. Real-Time Quantitative PCR (RT-qPCR)

Total RNA was isolated from ground frozen root tissues using Trizol® reagent (Ambion, Life Technology, Carlsbad, CA, USA) and treated with DNase. Afterwards, cDNA was synthesized from 2 µg of total RNAs using Superscript™ III reverse transcriptase (Invitrogen, Darmstadt, Germany), according to the manufacturer's protocol. PCR reactions were performed using SYBR Green PCR Master Mix and genes -specific primers and analyzed in a Step-OnePlus real-time PCR System (Applied Biosystems, Foster, CA, USA). Sequences of oligonucleotides used for expression of *EXP* and *XTH* genes were selected from the literature that have been shown to regulate cell expansion in rice seedlings [2,11]. *Ubiquitin* (Os03g13170) and *Actin* (Os03g50885) were used as internal standards [23,24]. The relative expression was normalized based on these two genes and expressed as relative values against rice seedlings under low nitrate condition. Relative transcript abundance was calculated using the ΔΔCT method [25]. The primers used for RT-qPCR are listed in Table S1.

2.6. Statistical Analysis

The experiments were designed in a completely randomized distribution. Two-way ANOVA ($p < 0.05$) model was applied to compare the means of the measured parameters with the factors selenium and nitrate concentration. The *F*-test was used to assess the differences between nitrate concentrations within each Se concentration and vice versa. All statistical analyses were performed using the R statistical software version 4.1.0.

3. Results

Nitrate increased elongation of rice shoot in a dose-dependent manner (Figure S1a). The maximum significant effect induced by nitrate occurred at 5.0 mM when shoot length was increased by 42% compared with very low nitrate condition (0.01 mM nitrate). On the other hand, the results revealed that primary root length of rice seedlings increased with increasing nitrate supply, reached the maximum value at optimal nitrate supply (0.05 mM nitrate), and then decreased when nitrate supply was further increased (Figure S1b). To characterize the effects of Se on seedlings growth in response to changes in nitrate concentration, rice seedlings were grown in nutrient solutions containing either 0- or 10-µM Se that were supplemented with 0.05 (low nitrate condition) or 5.0 mM nitrate (high nitrate condition) (Figure 1a). Selenium application to seedlings treated with low nitrate led to an

inhibition of primary root length (15%), length of lateral roots (30%), number of lateral roots (31%), root volume (40%), and root biomass (15%) compared with seedlings in 0.05 mM nitrate alone (Figure 1b–f). Moreover, primary root length, length of lateral roots, number of lateral roots, root volume, and root biomass increased in Se-treated seedlings grown under 5.0 mM nitrate compared with seedlings grown in 0.05 mM nitrate treated with Se, but not in seedlings treated with nitrate alone, leading to a N × Se interaction (Figure 1b–f). Shoot length and shoot biomass decreased in Se-treated seedlings under 0.05 mM and 5 mM nitrate compared with seedlings grown in nitrate alone (Figure 1g,h). On the other hand, shoot length and shoot biomass increased under 5 mM nitrate in both Se-treated seedlings and untreated ones compared with seedlings in 0.05 mM nitrate. In addition, there was a significant N × Se interaction for shoot/root ratio (Figure 1i). In this sense, there was a Se-dependent response for shoot/root ratio, which was modified by nitrate supply.

In order to verify the effect of Se treatment on biosynthesis of cytokinin and sugar, as well as on accumulation of nitrate and Se in response to nitrate, we measured zeatin, zeatin riboside, isopentenyladenine, soluble sugars, nitrate, and Se in both shoot and root of seedlings treated with Se grown under increasing nitrate supply. Compared with 0.05 mM nitrate, shoot concentrations of zeatin, zeatin riboside, and isopentenyladenine increased in both Se-untreated and Se-treated seedlings grown with 5 mM nitrate (Figure 2a–c). There was also a decrease in concentrations of zeatin and zeatin riboside in shoots of Se-treated seedlings compared with untreated ones in both nitrate conditions (Figure 2a,b). The concentrations of zeatin, zeatin riboside, and isopentenyladenine in the roots showed a significant N × Se interaction (Figure 2a–c). Compared with nitrate alone, cytokinin concentrations were significantly increased in the roots of Se-treated seedlings grown with 0.05 mM nitrate. However, Se combined with 5 mM nitrate decreased the concentrations of zeatin (39%), zeatin riboside (40%), and isopentenyladenine (47%) in the roots compared with seedlings grown under 5 mM nitrate (Figure 2a–c). Treatment with Se increased concentration of total sugars by 82% and 44% in shoot and root, respectively, compared with Se-untreated seedlings grown in 0.05 mM nitrate (Figure 2d). On the other hand, concentration of total sugars in shoot and root was not significantly affected by Se in 5 mM nitrate. A significant increase in nitrate concentration was observed in both shoot and root of seedlings grown in 5 mM nitrate compared with seedlings in 0.05 mM nitrate under both Se conditions (Figure 2e). Interestingly, shoot nitrate concentration showed a significant N × Se interaction, exhibiting a reduction in Se-treated seedlings compared with untreated ones in 5 mM nitrate. Selenite itself significantly increased concentration of total Se in shoot and root compared with selenite-untreated seedlings in both nitrate conditions (Figure 2f).

Given the evidence that the exposure of rice seedlings to Se in combination with increasing nitrate supply resulted in alteration in primary and lateral root elongation, we quantified mRNA expression of genes encoding proteins involved in cell expansion. In this context, cell expansion-related genes, including *OsEXP13*, *OsEXPA8*, *OsEXPA14*, *OsEXPB2*, *OsEXPB3*, *OsXTH2*, *OsXTH13*, and *OsXTH15* were upregulated in roots of rice seedlings treated with Se in 5 mM nitrate compared with Se combined with 0.05 mM nitrate, revealing N × Se interactions (Figure 3a–h). Under 0.05 mM nitrate, there was a downregulation in the expression of *OsEXPA8*, *OsEXPA14*, *OsEXPB2*, *OsEXPB3*, *OsXTH2*, *OsXTH13*, and *OsXTH15* in roots of Se-treated seedlings compared with untreated ones (Figure 3b–h). Moreover, the expression of *OsEXP13*, *OsEXPA8*, *OsEXPA14*, *OsXTH13*, and *OsXTH15* was induced in roots of Se-treated seedlings compared with untreated ones in 5 mM nitrate (Figure 3a–c,g,h). There was a decrease in the expression of *OsEXPA14* gene in roots of Se-untreated seedlings grown in 5 mM nitrate compared with 0.05 mM nitrate (Figure 3c). Treatments had no effect on expression of *OsXTH4* gene in roots of rice seedlings (Figure 3i).

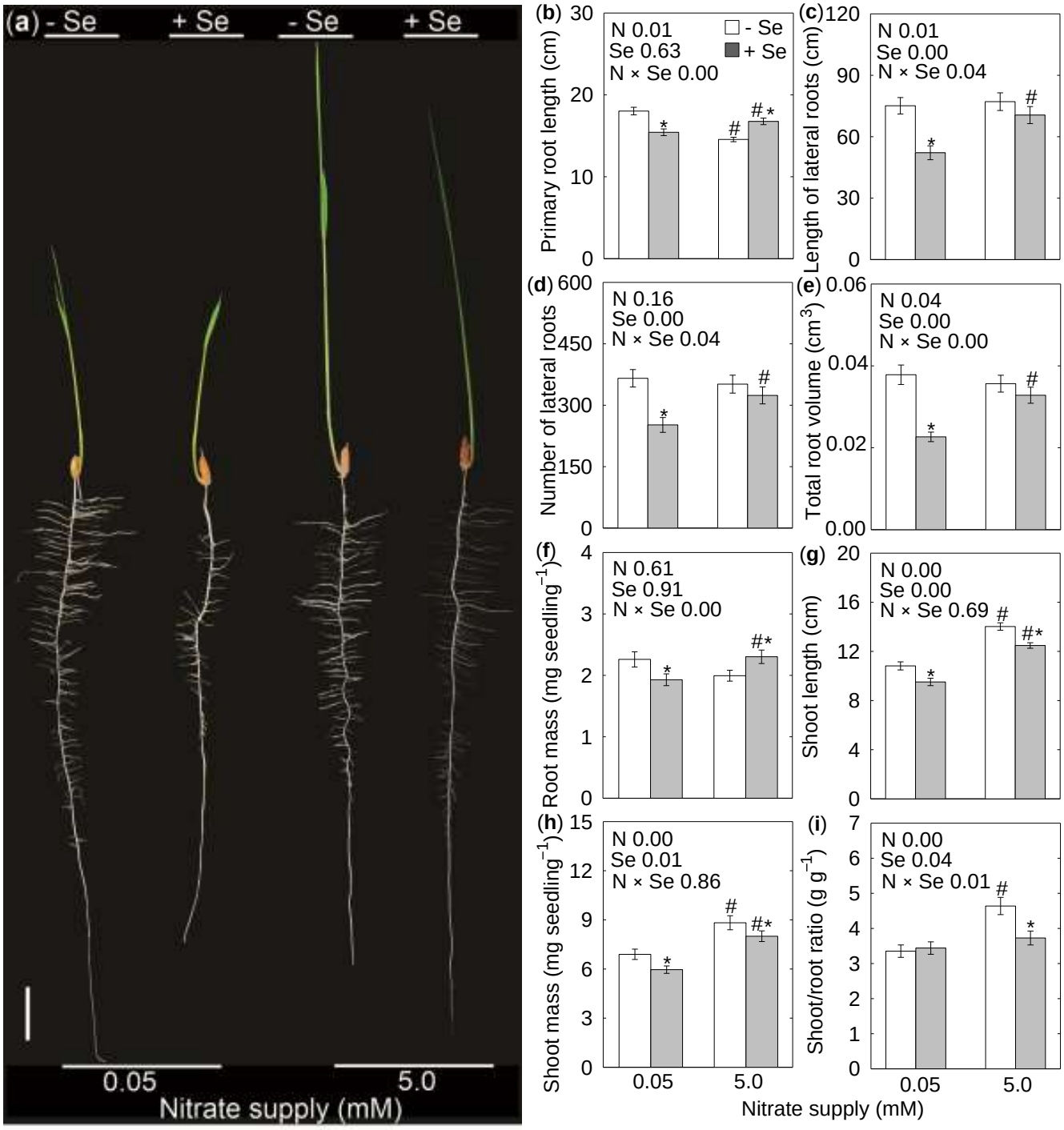

Figure 1. Effects of Se and nitrate treatments, singly and in combination, on growth of rice seedlings. (**a**) Phenotype of rice plants grown for 7 days under Se and nitrate treatments. The scale bar represents 2 cm. (**b**) Primary root length. (**c**) Total length of lateral roots. (**d**) Number of lateral roots. (**e**) Total root volume. (**f**) Root biomass. (**g**) Shoot length. (**h**) Shoot biomass. (**i**) Shoot/root ratio. Whitin each panel, the analysis of variance for the effect of nitrate (N) and selenium (Se) is shown along with the *p*-value, where a *p*-value smaller than 0.05 represents that the factor or both factors (N × Se) affects the outcome. Asterisks (*), when shown, indicate statistically different means between untreated and Se-treated plants within the same nitrate conditions ($p < 0.05$). Hashtags (#), when shown, indicate statistically different means between plants grown under 0.05 mM nitrate and 5 mM nitrate within the same Se treatment ($p < 0.05$). Data are mean ± standard error of three separate experiments, with five replicates of 10 seedlings each.

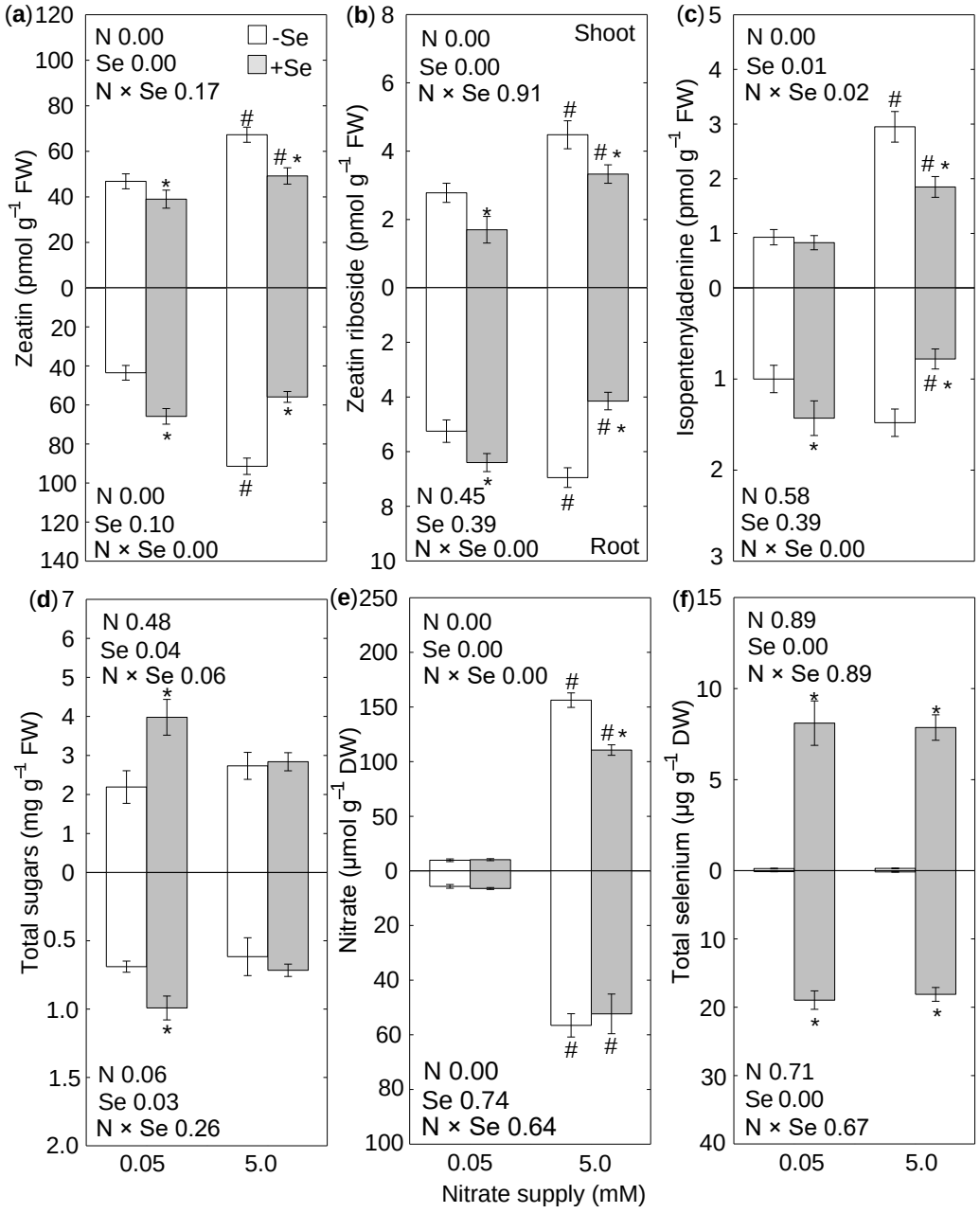

Figure 2. Effects of Se and nitrate treatments, singly and in combination, on concentrations of cytokinin, sugars, nitrate, and selenium in shoot and root of rice seedlings. (**a**) Zeatin. (**b**) Zeatin riboside. (**c**) N^6-(Δ^2-isopentenyl) adenine. (**d**) Total sugars. (**e**) Nitrate. (**f**) Total selenium. Whitin each panel, the analysis of variance for the effect of nitrate (N) and selenium (Se) is shown along with the *p*-value, where a *p*-value smaller than 0.05 represents that the factor or both factors (N × Se) affects the outcome. Asterisks (*), when shown, indicate statistically different means between untreated and Se-treated plants within the same nitrate conditions ($p < 0.05$). Hashtags (#), when shown, indicate statistically different means between plants grown under 0.05 mM nitrate and 5 mM nitrate within the same Se treatment ($p < 0.05$). Data are mean ± standard error of three separate experiments, with five replicates each.

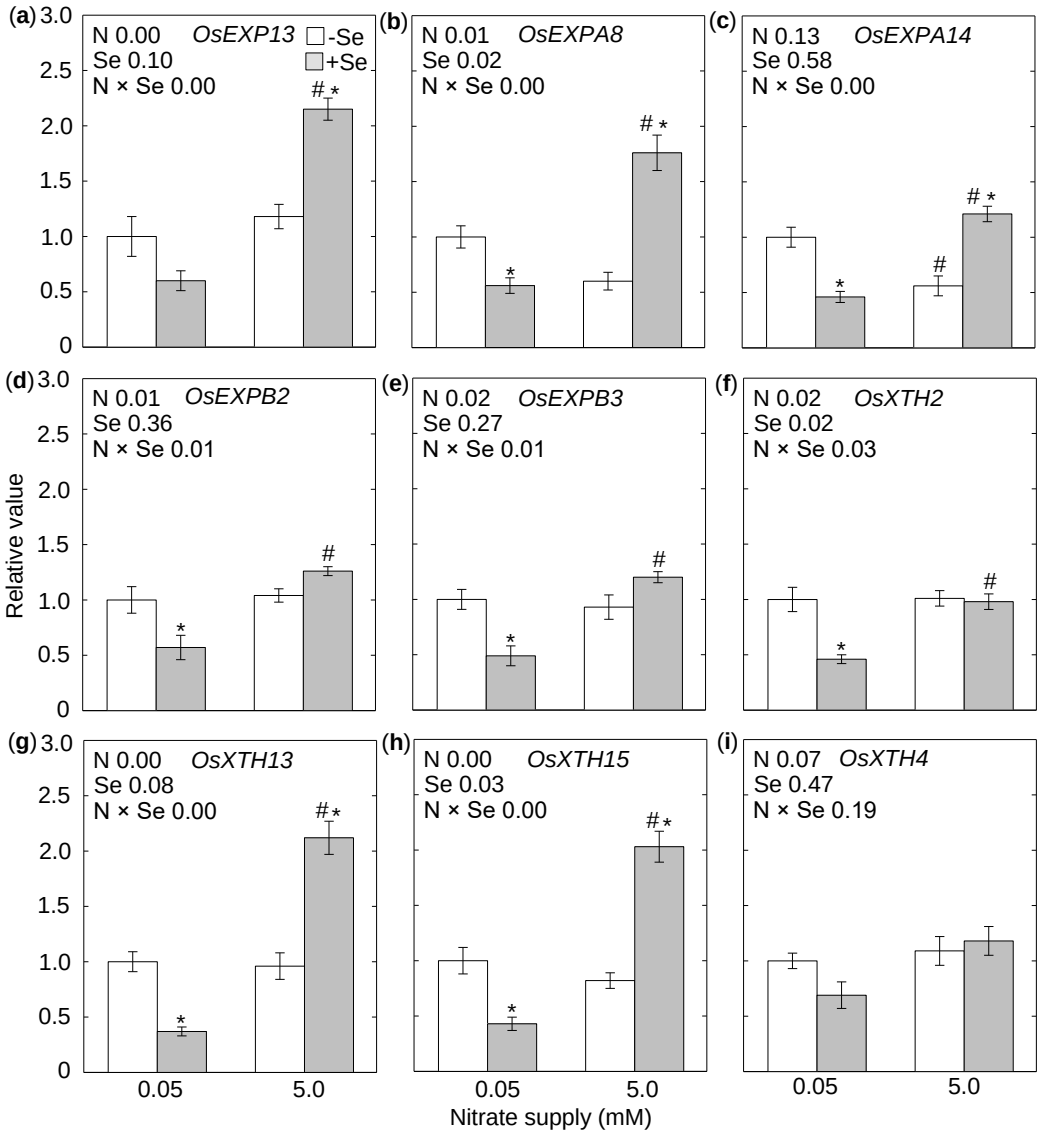

Figure 3. Effects of Se and nitrate treatments, singly and in combination, on expression of cell expansion-related genes in roots of rice seedlings. (**a**–**i**) Relative expression levels of *OsEXP13*, *OsEXPA8*, *OsEXPA14*, *OsEXPB2*, *OsEXPB3*, *OsXTH2*, *OsXTH13*, *OsXTH15*, and *OsXTH4*, in root. Whitin each panel, the analysis of variance for the effect of nitrate (N) and selenium (Se) is shown along with the *p*-value, where a *p*-value smaller than 0.05 represents that the factor or both factors (N × Se) affects the outcome. Asterisks (*), when shown, indicate statistically different means between untreated and Se-treated plants within the same nitrate conditions ($p < 0.05$). Hashtags (#), when shown, indicate statistically different means between plants grown under 0.05 mM nitrate and 5 mM nitrate within the same Se treatment ($p < 0.05$). Data are mean ± standard error of three separate experiments, with three replicates each.

4. Discussion

The availability of Se modifies root system architecture in rice seedlings, which is associated with the enhanced primary root elongation, and decreased lateral roots [11]. Root system architecture is also regulated by other minerals, including nitrogen, which has a positive effect in both cytokinin biosynthesis and signaling [26]. The results of the present study showed that Se supply to seedlings treated with low nitrate increased the cytokinin concentrations in the roots, and photosynthesis-derived sugars were responsible for the induction, a result that was associated with phenotypic changes in the root system. Moreover, Se application to seedlings treated with high nitrate resulted in an antagonistic

role of Se and nitrate in regulating cytokinin biosynthesis, which impacted shoot growth and root system architecture of rice seedlings.

It has been documented that reduced nitrogen availability inhibits cytokinin biosynthesis and stimulates primary root elongation of rice seedlings [2]. Consistent with this view, our analysis revealed that the primary root elongation increased in seedlings treated with low nitrate compared with high nitrate condition due to a decrease in concentrations of zeatin, and zeatin riboside, but not in roots of seedlings treated with Se grown on low nitrate condition (Figures 1 and 2). In this respect, Se application to seedlings treated with low nitrate increased cytokinin concentrations in the roots and decreased not only root size, but also number of lateral roots compared with seedlings grown in 0.05 mM nitrate alone (Figures 1 and 2). The decrease in cytokinin concentrations causes root cell elongation by increasing the expression of *EXP* and *XTH* genes in the roots of rice seedlings grown under nitrogen deficiency condition [2]. In our experimental setup, Se combined with 0.05 mM nitrate decreased the expression of *OsEXPA8*, *OsEXPA14*, *OsEXPB2*, *OsEXPB3*, *OsXTH2*, *OsXTH13*, and *OsXTH15* in roots compared with seedlings grown in 0.05 mM nitrate alone (Figure 3). These results imply that Se mediates downregulation of *EXP* and *XTH* genes through the induction of cytokinin biosynthesis in response to low nitrate availability. In addition, our study suggests that the effect of Se on cytokinin biosynthesis in roots of seedlings treated with low nitrate is dependent of changes in sugar concentrations in shoot, which in turn increased cytokinin concentration in the roots (Figure 2). This finding is consistent with previous reports that photosynthetic generated sugars in the leaves cause increased in cytokinin biosynthesis in the roots of *Arabidopsis* plants [27,28]. Interestingly, seedlings growth with 0.05 mM nitrate associated with Se fertilization have similar shoot/root ratios and decreased concentrations of zeatin and zeatin riboside in the shoot compared with seedlings in 0.05 mM nitrate alone (Figures 1 and 2). Thus, it is likely that the shoot/root ratio only increases when cytokinin accumulates in the shoot, and that this response can be separated from the effect of sugar concentration in the shoot. In fact, the higher shoot/root ratio of seedlings non-treated with Se compared with seedlings treated with Se grown with 5 mM nitrate was related to the accumulation of zeatin, zeatin riboside, and isopentenyladenine in the shoot (Figures 1 and 2).

Application of nitrate to the roots acts as a signal to induce cytokinin signaling in the leaves [29]. Our results indicated that accumulation of nitrate and cytokinin in the shoot of rice seedlings grown on 5 mM was accompanied by inhibition of primary root elongation and an increase in the shoot/root ratio compared with seedlings treated with Se combined with increasing nitrate supply (Figures 1 and 2). It has been pointed out that nitrate accumulation in the shoots of tobacco plants was positively correlated with an inhibition of root growth, and an increase in the shoot/root ratio [8]. It seems possible, therefore, that Se altered the nitrate accumulation and cytokinin biosynthesis in rice seedlings grown under high nitrate condition. Consistent with this observation, rice seedlings treated with Se grown under 5 mM nitrate showed shoot growth decreased accompanying a reduction in concentrations of zeatin, zeatin riboside, and isopentenyladenine in the shoot and root, whereas it exhibited an increase in root biomass compared with seedlings supplied 5 mM nitrate (Figures 1 and 2). Moreover, Se application to seedlings treated with high nitrate resulted in upregulation of *OsEXP13*, *OsEXPA8*, *OsEXPA14*, *OsXTH13*, and *OsXTH15* in the roots compared with seedlings under 5 mM nitrate, which in turn increased root size (Figures 1 and 3). Thus, we propose that upregulation of *EXP* and *XTH* genes in the roots of seedlings grown under high nitrate supply is mediated by Se through inhibition of cytokinin biosynthesis. In fact, concentrations of zeatin, zeatin riboside, and isopentenyladenine in the rice roots increased with Se treatment under low nitrate concentration, but not under high nitrate condition, due to Se × nitrate interactions (Figure 2). In this sense, there was a Se-dependent response for accumulation of cytokinin in the roots of rice seedlings, which was modified by nitrate supply. Together, these findings suggest that the reduction in shoot size due to Se treatment under high nitrate condition may be attributed to the lower cytokinin translocation between the root and shoot.

Our results are integrated in a model presented in Figure 4 in which low nitrate condition causes a decrease in cytokinin concentrations in the roots and reduces translocation to shoot. Thus, the inhibition of cytokinin accumulation in the root and shoot by low nitrate condition increases primary root elongation and decreases shoot size of rice seedlings. Moreover, Se plus low nitrate concentration leads to increased sugar concentration in the shoot and root, an increased cytokinin concentrations in the root and inhibited absolute rate of root growth. On the other hand, high nitrate condition increases cytokinin biosynthesis in the root and translocation to shoot, leading to decreased in primary root elongation and increased in shoot size. In addition, Se negatively regulates the cytokinin biosynthesis in the roots of rice seedlings grown under high nitrate condition, and thereby decreasing cytokinin translocation to shoot. Thus, the reduction in cytokinin concentrations by Se plus high nitrate decreases shoot size, while increasing primary root elongation and total root biomass. Interestingly, Se and nitrogen fertilization improves the nutritional composition of rice grains [30]. This, together with the fact that Se combined with nitrate affects rice seedlings growth, suggests a prominent role for Se and nitrogen in enhancing rice plant performance.

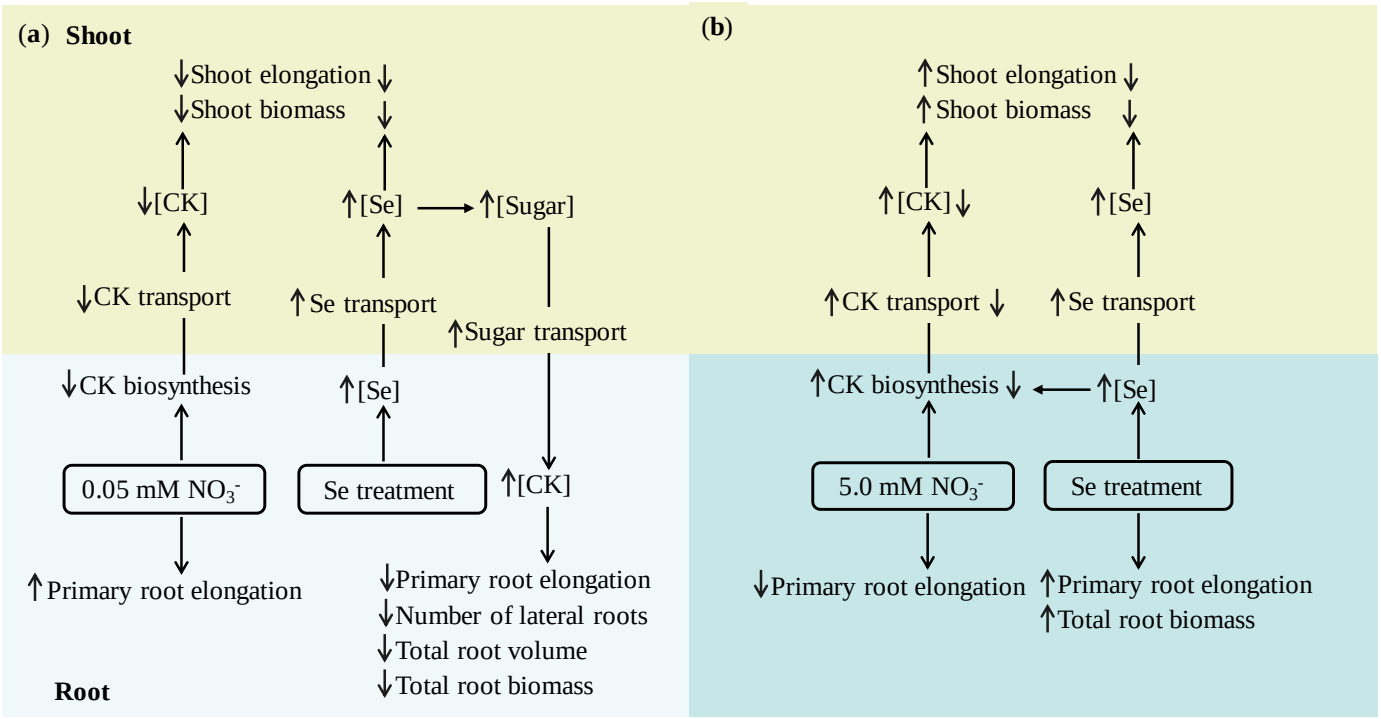

Figure 4. Scheme summarizing the effects of Se and nitrate treatments, singly and in combination, on growth of rice seedlings. Diagrams of rice seedlings grown under low nitrate condition (**a**) and high nitrate condition (**b**) singly and in combination with Se. Treatment of rice seedlings with 0.05 mM nitrate decreases cytokinin biosynthesis in the shoot and root, which may then increase primary root elongation while decrease shoot size compared with seedlings grown in 5 mM nitrate. Moreover, Se application to seedlings treated with low nitrate increase sugar concentrations in the shoot and root and increases cytokinin concentrations in the root, while decreasing root size compared with seedlings in 0.05 mM nitrate alone. On the other hand, seedlings grown on 5 mM nitrate have a high cytokinin concentrations in root and shoot, the shoot size increases, and primary root elongation decreases compared with seedlings in 0.05 mM nitrate. In addition, Se negatively regulates the cytokinin biosynthesis in the roots of rice seedlings grown under high nitrate condition, and thereby decreasing cytokinin translocation to shoot compared with seedlings in 5 mM nitrate alone. The reduction in cytokinin concentrations by Se under high nitrate condition decreases shoot size, while increasing root size.

5. Conclusions

In summary, Se application to seedlings treated with low nitrate decreases root and shoot size through alteration of the sugar and cytokinin balance. Accumulation of sugar in the shoot under Se application to seedlings treated with low nitrate is likely to be related to reduction in root growth, in which stimulation of cytokinin biosynthesis and sugar accumulation in the roots are linked. In this case, our results indicate that Se regulates sugar metabolism and cytokinin biosynthesis to cope with low nitrate condition. The increasing nitrate supply induces cytokinin biosynthesis in the roots, whereas Se results in the opposite effects. Therefore, Se increases root size of rice seedlings probably occurs through its inhibition of cytokinin biosynthesis. In this case, the decreases in cytokinin concentrations in the roots are associated with an increase in the expression of cell expansion-related genes, including *OsEXP13*, *OsEXPA8*, *OsEXPA14*, *OsXTH13*, and *OsXTH15*, which in turn changes in root architecture in rice seedlings. Moreover, Se decreases shoot size of rice seedlings growth under high nitrate condition by decreasing the cytokinin translocation between root and shoot.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/nitrogen5010013/s1, Figure S1: Effect of nitrate on shoot and root of rice seedlings; Table S1: Primers sequences used for real-time PCR.

Author Contributions: T.A.L.M., C.S.A., G.A.S. and L.S.T. conducted experiments and statistical analysis. T.A.L.M., V.A.A., D.J.C.L., D.M.R. and L.S.T. performed literature survey. C.S.A., G.A.S., L.S.T., D.M.R., V.A.A. and D.J.C.L. designed the research and interpreted the results. All authors contributed to the writing of the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Foundation for Research Assistance of Minas Gerais State, Brazil (FAPEMIG, grants BPD-00823-22 and RED-00053-16), the National Institute of Science and Technology in Plant Physiology under Stress Conditions (INCT, Plant Stress Physiology, grant 406455/2022-8) and the National Council for Scientific and Technological Development, Brazil (CNPq, grant 303230/2022-3).

Data Availability Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Acknowledgments: This study was financed in part by the Brazilian Federal Agency for Support and Evaluation of Graduate, Brazil (CAPES, Finance Code 001). Research fellowship awarded by CNPq-Brazil to DMR is also gratefully acknowledged.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Ye, J.Y.; Tian, W.H.; Jin, C.W. Nitrogen in plants: From nutrition to the modulation of abiotic stress adaptation. *Stress Biol.* **2022**, *2*, 4. [CrossRef] [PubMed]
2. Wang, Q.; Zhu, Y.; Zou, X.; Li, F.; Zhang, J.; Kang, Z.; Li, X.; Yin, C.; Lin, Y. Nitrogen Deficiency-Induced Decrease in Cytokinins Content Promotes Rice Seminal Root Growth by Promoting Root Meristem Cell Proliferation and Cell Elongation. *Cells* **2020**, *9*, 916. [CrossRef]
3. Pavlů, J.; Novák, J.; Koukalová, V.; Luklová, M.; Brzobohatý, B.; Černý, M. Cytokinin at the Crossroads of abiotic stress signalling pathways. *Int. J. Mol. Sci.* **2018**, *19*, 2450. [CrossRef] [PubMed]
4. Shah, S.; Cai, L.; Li, X.; Fahad, S.; Wang, D. Influence of cultivation practices on the metabolism of cytokinin and its correlation in rice production. *Food Energy Secur.* **2023**, *12*, 488. [CrossRef]
5. Poitout, A.; Crabos, A.; Petřík, I.; Novák, O.; Krouk, G.; Lacombe, B.; Ruffel, S. Responses to systemic nitrogen signaling in Arabidopsis roots involve trans-zeatin in shoots. *Plant Cell* **2018**, *30*, 1243–1257. [CrossRef]
6. Reed, R.C.; Bradford, K.J.; Khanday, I. Seed germination and vigor: Ensuring crop sustainability in a changing climate. *Heredity* **2022**, *128*, 450–459. [CrossRef] [PubMed]
7. Lynch, J.P.; Galindo-Castañeda, T.; Schneider, H.M.; Sidhu, J.S.; Rangarajan, H.; York, L.M. Root phenotypes for improved nitrogen capture. *Plant Soil* 2023, *Advance online publication*. [CrossRef]
8. Scheible, W.-R.; Lauerer, M.; Schulze, E.-D.; Caboche, M.; Stitt, M. Accumulation of nitrate in the shoot acts as a signal to regulate shoot-root allocation in tobacco. *Plant J.* **1997**, *11*, 671–691. [CrossRef]

9. Puig, J.; Pauluzzi, G.; Guiderdoni, E.; Gantet, P. Regulation of shoot and root development through mutual signaling. *Mol. Plant* **2012**, *5*, 974–983. [CrossRef]
10. Hawkesford, M.J.; Cakmak, I.; Coskun, D.; De Kok, L.J.; Lambers, H.; Schjoerring, J.; Branco, F.J. Functions of macronutrients. In *Marschner's Mineral Nutrition of Plants*, 4th ed.; Rengel, Z., Cakmak, I., White, P.J., Eds.; Elsevier: London, UK, 2023; pp. 201–281. [CrossRef]
11. Malheiros, R.S.P.; Costa, L.C.; Ávila, R.T.; Pimenta, T.M.; Teixeira, L.S.; Brito, F.A.L.; Zsögön, A.; Araújo, W.L.; Ribeiro, D.M. Selenium downregulates auxin and ethylene biosynthesis in rice seedlings to modify primary metabolism and root architecture. *Planta* **2019**, *250*, 333–345. [CrossRef]
12. Ribeiro, D.M.; Silva Júnior, D.D.; Cardoso, F.B.; Martins, A.O.; Silva, W.A.; Nascimento, V.L.; Araújo, W.L. Growth inhibition by selenium is associated with changes in primary metabolism and nutrient levels in Arabidopsis thaliana. *Plant Cell Environ.* **2016**, *39*, 2235–2246. [CrossRef]
13. Dimkovikj, A.; Van Hoewyk, D. Selenite activates the alternative oxidase pathway and alters primary metabolism in Brassica napus roots: Evidence of a mitochondrial stress response. *BMC Plant Biol.* **2014**, *14*, 259. [CrossRef]
14. Lima, L.W.; Pilon-Smits, E.A.H.; Schiavon, M. Mechanisms of selenium hyperaccumulation in plants: A survey of molecular, biochemical and ecological cues. *BBA—Gen. Subj.* **2018**, *1862*, 2343–2353. [CrossRef]
15. Wang, K.; Wang, Y.; Li, K.; Wan, Y.; Wang, Q.; Zhuang, Z.; Guo, Y.; Li, H. Uptake, translocation and biotransformation of selenium nanoparticles in rice seedlings (*Oryza sativa* L.). *J. Nanobiotechnology* **2020**, *18*, 103. [CrossRef]
16. Lehotai, N.; Kolbert, Z.; Peto, A.; Feigl, G.; Ordog, A.; Kumar, D.; Tari, I.; Erdei, L. Selenite-induced hormonal and signalling mechanisms during growth of *Arabidopsis thaliana* L. *J. Exp. Bot.* **2012**, *63*, 5677–5687. [CrossRef] [PubMed]
17. Malheiros, R.S.P.; Gonçalves, F.C.M.; Brito, F.A.L.; Zsögön, A.; Ribeiro, D.M. Selenomethionine induces oxidative stress and modifies growth in rice (*Oryza sativa* L.) seedlings through effects on hormone biosynthesis and primary metabolism. *Ecotoxicol. Environ. Saf.* **2020**, *189*, 109942. [CrossRef] [PubMed]
18. Hoagland, D.R.; Arnon, D.I. *The Water Culture Method for Growing Plants Without Soils*; California Agricultural Experimental Station: Berkeley, CA, USA, 1950.
19. Zhu, J.; Kaeppler, S.; Lynch, J.P. Mapping of QTLs for lateral root branching and length in maize (*Zea mays* L.) under differential phosphorus supply. *Theor. Appl. Genet.* **2005**, *111*, 688–695. [CrossRef] [PubMed]
20. El Mehdawi, A.F.; Jiang, Y.; Guignardi, Z.S.; Esmat, A.; Pilon, M.; Pilon-Smits, E.A.H.; Schiavon, M. Influence of sulfate supply on selenium uptake dynamics and expression of sulfate/selenate transporters in selenium hyperaccumulator and non-hyperaccumulator Brassicaceae. *New Phytol.* **2018**, *217*, 194–205. [CrossRef] [PubMed]
21. Cross, J.M.; von Korff, M.; Altmann, T.; Bartzetko, L.; Sulpice, R.; Gibon, Y.; Palacios, N.; Stitt, M. Variation of enzyme activities and metabolite levels in 24 Arabidopsis accessions growing in carbon-limited conditions. *Plant Physiol.* **2006**, *142*, 1574–1588. [CrossRef] [PubMed]
22. Song, W.; Li, J.; Sun, H.; Huang, S.; Gong, X.; Ma, Q.; Zhang, Y.; Xu, G. Increased photosynthetic capacity in response to nitrate is correlated with enhanced cytokinin levels in rice cultivar with high responsiveness to nitrogen nutrients. *Plant Soil.* **2013**, *373*, 981–993. [CrossRef]
23. Li, X.; Yang, D.-L.; Sun, L.; Li, Q.; Mao, B.; He, Z. The systemic acquired resistance regulator OsNPR1 attenuates growth by repressing auxin signaling through promoting IAA-Amido synthase expression. *Plant Physiol.* **2016**, *172*, 546–558. [CrossRef]
24. Figueiredo, D.D.; Barros, P.M.; Cordeiro, A.M.; Serra, T.S.; Lourenço, T.; Chander, S.; Oliveira, M.M.; Saibo, N.J. Seven zinc-finger transcription factors are novel regulators of the stress responsive gene OsDREB1B. *J. Exp. Bot.* **2012**, *63*, 3643–3656. [CrossRef]
25. Livak, K.J.; Schmittgen, T.D. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods* **2001**, *25*, 402–408. [CrossRef] [PubMed]
26. Kamada-Nobusada, T.; Makita, N.; Kojima, M.; Sakakibara, H. Nitrogen-dependent regulation of de novo cytokinin biosynthesis in rice: The role of glutamine metabolism as an additional signal. *Plant Cell Physiol.* **2013**, *54*, 1881–1893. [CrossRef] [PubMed]
27. Kiba, T.; Takebayashi, Y.; Kojima, M.; Sakakibara, H. Sugar-induced de novo cytokinin biosynthesis contributes to Arabidopsis growth under elevated CO_2. *Sci. Rep.* **2019**, *9*, 7765. [CrossRef] [PubMed]
28. Sakakibara, H. Cytokinin biosynthesis and transport for systemic nitrogen signaling. *Plant J.* **2021**, *105*, 421–430. [CrossRef]
29. Sakakibara, H.; Hayakawa, A.; Deji, A.; Gawronski, S.W.; Sugiyama, T. His-Asp phosphotransfer possibly involved in the nitrogen signal transduction mediated by cytokinin in maize: Molecular cloning of cDNAs for two-component regulatory factors and demonstration of phosphotransfer activity in vitro. *Plant Mol. Biol.* **1999**, *41*, 563–573. [CrossRef]
30. Teixeira, L.S.; Pimenta, T.M.; Brito, F.A.L.; Malheiros, R.S.P.; Arruda, R.S.; Araújo, W.L.; Ribeiro, D.M. Selenium uptake and grain nutritional quality are affected by nitrogen fertilization in rice (*Oryza sativa* L.). *Plant Cell Rep.* **2021**, *40*, 871–880. [CrossRef]