Journal of Plant Physiology 268 (2022) 153586

Contents lists available at ScienceDirect

Journal of Plant Physiology

journal homepage: www.elsevier.com/locate/jplph

OsGF14b is involved in regulating coarse root and fine root biomass partitioning in response to elevated [CO_2] in rice

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ARTICLE INFO

Keywords:
OsGF14b
Elevated [CO_2]
Root biomass
Root partitioning
Rice

ABSTRACT

Elevated [CO_2] can increase rice biomass and yield, but the degree of this increase varies substantially among cultivars. Little is known about the gene loci involved in the acclimation and adaptation to elevated [CO_2] in rice. Here, we report on a T-DNA insertion mutant in japonica rice exhibiting a significantly enhanced response to elevated [CO_2] compared with the wild type (WT). The root biomass response of the mutant was higher than that of the WT, and this manifested in the number of adventitious roots, the average diameter of roots, and total root length. Furthermore, coarse roots (>0.6 mm) and thin lateral roots (<0.2 mm) were more responsive to elevated [CO_2] in the mutant. When exposed to lower light intensity, however, the response of the mutant to elevated [CO_2] was not superior to that of the WT, indicating that the high response of the mutant under elevated [CO_2] was dependent on light intensity. The T-DNA insertion site was located in the promoter region of the *OsGF14b* gene, and insertion resulted in a significant decrease in *OsGF14b* expression. Our results indicate that knockout of *OsGF14b* may improve the response to elevated [CO_2] in rice by enhancing carbon allocation to coarse roots and to fine lateral roots.

1. Introduction

Since the industrial revolution, the atmospheric CO_2 concentration has risen to exceed 400 μmol mol^{-1} in 2017 (Dlugokencky and Tans, 2018), and is expected to continue to increase and may well exceed values of 700 μmol mol^{-1} by 2100 (Coskun et al., 2016; Thompson et al., 2017). As the substrate for photosynthesis in crops, CO_2 has a fundamental impact on crop growth and development and on nearly all physiological and biochemical processes (Kim et al., 2001; Zhang et al., 2015). As the world's human population is expected to reach ten billion people by 2050, to ensure food security, it is crucial to better understand crop growth responses to elevated [CO_2], an understanding critical to help breeders improve crop germplasm resources that can be deployed in response to climate change.

Rice (*Oryza sativa* L.) is a major staple food crop for more than half of the world's population (Min et al., 2021). In response to elevated [CO_2], rice yield improves as a consequence of increased plant growth, tiller number, and leaf area (Ainsworth et al., 2008; Kim et al., 2001; Zhu et al., 2013). In addition to the direct effects of CO_2 on leaf photosynthesis, the root system also shows a dramatic response to elevated [CO_2], however (Thompson et al., 2017; Wu et al., 2018). A positive relationship has been reported between root dry matter and crop N uptake in the rice cultivar Akitakomachi under free-air CO_2 enrichment (FACE) conditions (Kim et al., 2001). Using four rice cultivars, Zhu et al. (2013) found that root biomass (instead of root number) and bending strength were increased by 12–38% under elevated [CO_2]. Root branching, root length, and root diameter are the three key determinants of root surface area, which, in turn, is critical to nutrient uptake (Atkinson et al., 2014; Di et al., 2021; Li et al., 2015). Elevated [CO_2] has been reported to exert positive effects on all three components (Day et al., 1996; Jongen et al.,

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https://doi.org/10.1016/j.jplph.2021.153586
Received 17 November 2021; Received in revised form 30 November 2021; Accepted 30 November 2021
Available online 5 December 2021

1995). When CO_2 supply is sufficient, light intensity can become limiting to photosynthesis and is known to affect plant morphology profoundly (Hubbart et al., 2012). However, root morphological changes under elevated [CO_2] have not been studied in detail.

At the physiological and biochemical levels, CO_2 enrichment produces significant effects on leaf photosynthesis, carbohydrate transport, and protein phosphorylation (Ainsworth et al., 2004; Dineshram et al., 2013; Long et al., 2004; Zhu et al., 2014). Recent studies have explored the molecular mechanisms involved in plant adaptation to elevated [CO_2]. Few genes have been successfully and specifically implicated in the response to elevated [CO_2] in crops, including rice, however. Kanno et al. (2017) reported a small decrease in Rubisco content, achieved by suppressing the RBCS multigene family, can lead to increases in photosynthesis and biomass deposition at elevated [CO_2]. In addition, *OsRab6a*, which encodes a small GTPase, is involved in the regulation of rice growth, grain yield, and accumulation of iron in response to elevated [CO_2] (Yang et al., 2020). The G protein γ subunit *qPE9-1* is also associated with rice adaptation to elevated [CO_2] by regulating leaf photosynthesis (Wang et al., 2021). Despite several of these associations, the key genetic locus governing the rice root response under elevated [CO_2] needs to as yet be identified, and its identification could pave the way to improving crop yield responses to elevated [CO_2] through genetic manipulation.

It is well established that 14-3-3 proteins take part in root growth and several key physiological roots processes, including carbon and nitrogen uptake and utilization and proton-flow dynamics that govern root growth (Comparot et al., 2003; Sato et al., 2011; Schoonheim et al., 2007; Wang et al., 2014, 2016). Some studies have shown that 14-3-3 proteins can target sucrose phosphate synthase (SPS) and adjust its activity (Bornke, 2005; Schoonheim et al., 2007). In *Arabidopsis*, 14-3-3 proteins play important roles in root growth (Mayfield et al., 2012). In barley, the small subunit of Rubisco has also been reported as a target of a 14-3-3 protein in barley (Schoonheim et al., 2007). The *OsGF14b* gene belongs to the rice 14-3-3 protein family, which is comprised of eight members. It was previously reported that *OsGF14b* plays varying roles in panicle and leaf blast resistance (Liu et al., 2016), and functions in rice drought and osmotic resistance have also been demonstrated (Liu et al., 2019). In addition, *OsGF14b* can induce the expression of IAA-synthesis-related proteins (Yan et al., 2021).

In this study, we identified a rice mutant with the potential for a strong response to elevated [CO_2]. The objectives of our study were: (1) to investigate the mutation site and gene expression status in this mutant; (2) to characterize the shoot and root response to elevated [CO_2] in the mutant; (3) to examine the root system attributes responsible for the CO_2 enhancement; and (4) to clarify the relationship between the root system response and ambient light intensity. The exploration of these mechanisms is essential for the establishment of a theoretical basis for rice's carbon-use efficiency and its yield response under elevated [CO_2].

2. Material and methods

2.1. Plant material and growth conditions

The WT japonica rice line, Dongjin, and a series of T-DNA insertion lines were purchased from the mutant library of the Crop Biotech Institute, Department of Plant Systems Biotech, Kyung Hee University, Republic of Korea (http://cbi.khu.ac.kr/RISD_DB.html). Through screening, a rice mutant line with the potential for a strong response to elevated [CO_2] was obtained. Hydroponic experiments were carried out in two controlled-environment chambers with adjustable CO_2 concentration. Each chamber was equipped with an infrared CO_2 sensor, capable of passing the CO_2 concentration signal to a central controller to adjust the CO_2 concentration in the chamber. The chamber was connected to a CO_2 gas cylinder. For the experiment, the CO_2 concentration in the two incubators was 380 ppm (control) and 760 ppm (treatment), respectively. Photoperiod was set to 12 h day/12 h night, relative humidity was set to 80%, and temperature was set to 30 °C day/25 °C night. The hydroponic nutrient solution was prepared and modified referring to the formula developed by the International Rice Research Institute (IRRI). The composition of the nutrient solution was as follows: 1.25 mM NH_4NO_3,0.3 mM KH_2PO_4,0.35 mM K_2SO_4,1 mM $CaCl_2{\cdot}2H_2O$,1 mM $MgSO_4{\cdot}7H_2O$,0.5 mM $Na_2SiO_3{\cdot}9H_2O$,9 μM $MnCl_2{\cdot}4H_2O$,0.39 μM $Na_2MoO_4{\cdot}2H_2O$, 20 μM H_3BO_3,0.77 μM $ZnSO_4{\cdot}7H_2O$,0.32 μM $CuSO_4{\cdot}5H_2O$, 20 μM EDTA-Fe ($FeSO_4{\cdot}7H_2O$ + Na_2-EDTA). Seedlings were placed in 2-L hydroponic tanks, with four plants per tank, and were maintained in the controlled-environment chambers for two weeks.

2.2. Sample handling and collection

Seeds of uniform size were selected and disinfected with 10% H_2O_2 for 30 min, washed five times with deionized water, soaked in deionized water at 30 °C for one day, and germination occurred in darkness for 48 h. The germinated seeds were selected and planted. After three days of culture in one-fourth strength nutrient solution, and seven days of culture in half-strength nutrient solution, seedlings of identical height were selected and treated in different chambers. The full-strength nutrient solution was replaced every two days during the treatment lasting for two weeks. Samples were collected and divided into shoots and roots. Shoot samples were heated at 105 °C for 30 min and then dried at 80 °C for dry weight measurement. Root samples were measured for dry weight following root morphology analysis. The root morphology was scanned with a root scanner, and data were analyzed with the root analysis software WinRHIZO 2012 (Chen et al., 2020). The light source was metal halogen lamp and high pressure sodium lamp to form a balanced plant growth spectrum (PERCIVAL, USA). Light treatment was carried out with normal and with lowered light intensities. The light intensity at plant height was approximately 1200 μmol m^{-2} s^{-1} and 170 μmol m^{-2} s^{-1}, respectively. For determination of relative expression of the *OsGF14b* gene in different tissues, samples were collected at different periods of plant growth. Roots, younger leaves, and stems were collected from seedlings at two weeks, old leaves, younger panicles, and panicles during the flowering period were collected from the booting stage to the flowering stage. Samples were placed in liquid nitrogen immediately after collection and then frozen in a −80 °C freezer for use.

2.3. Identification of insertion sites and quantitative PCR

Since T-DNA insertion mutants had long fragment insertions, amplification of normal-size fragments was only possible in the wild type. For the mutant, primers were designed on one or both sides of the boundary of the insertion vector, and on both sides of the original genome insertion site. Amplification of the band containing a fragment from the vector is thus possible for the mutant, while, for the WT, amplification of the band did not occur as there was no inserted fragment present. The primers for identification were: LP-2, ATTTTGCCAGACGTTTGTCC, RP-2, AGCAAACATGGACCAGAACC, Tail-R1, ATGGAACTCACCTGGTACCTGG. Total RNA was isolated with the Total plant RNA rapid extraction kit (Sangon Biotech Co., Ltd, Shanghai, China). ChamQ SYBR qPCR Master Mix (Vazyme Biotech Co., Ltd, Nanjing, China) was used in real-time quantitative PCR. The primers for qPCR were: Ubiqitin-F, CGCAAGTACAACCAGGACAAGATG, Ubiqitin-R, CCAGGGAGATAACAACGGAAGC, P1–F, CCTGAGCGAGGAGTCC TACA, P1-R, CTGATCTCT TCCGCGGTGTC.

2.4. Data processing

Analysis of variance was performed for all data using EXCEL2016 and SPSS analysis software. Real-time quantitative PCR data were analyzed according to the method proposed by Pfaffl et al. (Pfaffl, 2001). Sigmaplot 12.5 was used for plot generation.

3. Results

3.1. The mutant is a homozygous T-DNA insertion mutant

In order to explore the mechanisms of the response to elevated [CO_2], we screened a series of T-DNA insertion lines under elevated [CO_2] conditions, and identified a rice mutant with the potential for a strong response to elevated [CO_2]. To investigate whether the mutant was homozygous, we first used the primers designed from the genome and the broader of the T-DNA fragments, LP-2, RP-2 and Tail-R1 (Fig. 1A). As shown in Fig. 1B, the WT had a fragment amplified by primers LP-2 and RP-2 (line 2), while the mutant possessed a fragment amplified by primers Tail-R1 and RP-2 (line 3, 5, 7), indicating that the mutant is indeed homozygous. The BLAST results showed that the T-DNA fragment was inserted in the site approximately 750bp outside the first exon of the genome, the promoter region. The *Os04g0462500* gene identified here, named *OsGF14b*, contained five exons and four introns, and is a member of the 14-3-3 protein family in rice. Compared with the expression level in the WT, the mutant showed a 90% lower expression level of *OsGF14b* (Fig. 1C), indicating that the insertion significantly suppressed the expression of this gene.

To understand how *OsGF14b* is regulated, the expression of *OsGF14b* was investigated in different tissues of the WT under normal growth conditions. As shown in Fig. 2, the highest expression was observed in the stem, with considerable expression in roots and younger panicles, and the lowest expression in the panicle during the flowering period.

3.2. Root biomass of the mutant is more responsive to elevated [CO_2]

In general, the mutant showed better growth compared to the WT (Fig. 3A and B). In comparison to control [CO_2] conditions, the response of the shoot biomass of the mutant to elevated [CO_2] was significantly stronger than that of the WT, with an increase of 33.3% in the WT and of 53.3% in the mutant (Fig. 3C). For root biomass, the difference was more pronounced under elevated [CO_2], with an increase of 69.9% in the mutant and 42.1% in the WT (Fig. 3D).

3.3. Root architecture of the mutant is more responsive under increased [CO_2]

The root morphological characteristics, such as the numbers of adventitious roots and root thickness, were further examined to probe the nature of the increase in root biomass. In comparison to the increase of 16.4% in the WT under elevated [CO_2], the mutant showed a higher increase of 46.2% in the number of adventitious roots (Fig. 4A). The mean root diameter of the mutant also increased 58.3% under elevated [CO_2], while that of the WT increased by 32.6% (Fig. 4B). The results show a better response in the mutant in terms of the number of adventitious roots and root thickness under elevated [CO_2].

In addition, the root surface area of the WT increased by 32.8% under increased [CO_2], while that of the mutant showed a more substantial increase of 47.3% (Fig. 5A). Compared with control [CO_2] conditions, the stimulation of the root volume was also larger in the mutant (increase by 75.0%), than in the WT (increase by 57.9%) under elevated [CO_2] (Fig. 5B). This change in root surface area was then also reflected in the total root length, which significantly increased under elevated [CO_2], with an enhancement of 28.4% in the mutant and 13.3% in the WT (Fig. 3D).

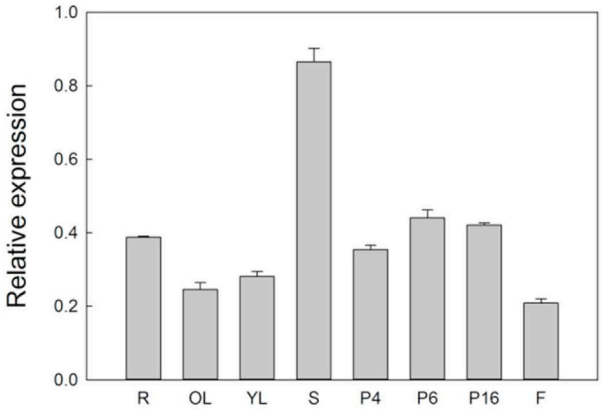

Fig. 2. Relative expression of the *OsGF14b* gene in different tissues of the WT The gene expression in roots (R), old leaves (OL), younger leaves (YL), stems (S), younger panicles (P4, P6, and P16, respectively, refer to panicle lengths of 4 cm, 6 cm, and 16 cm), and panicles during the flowering period (F). Error bars indicate ± SD.

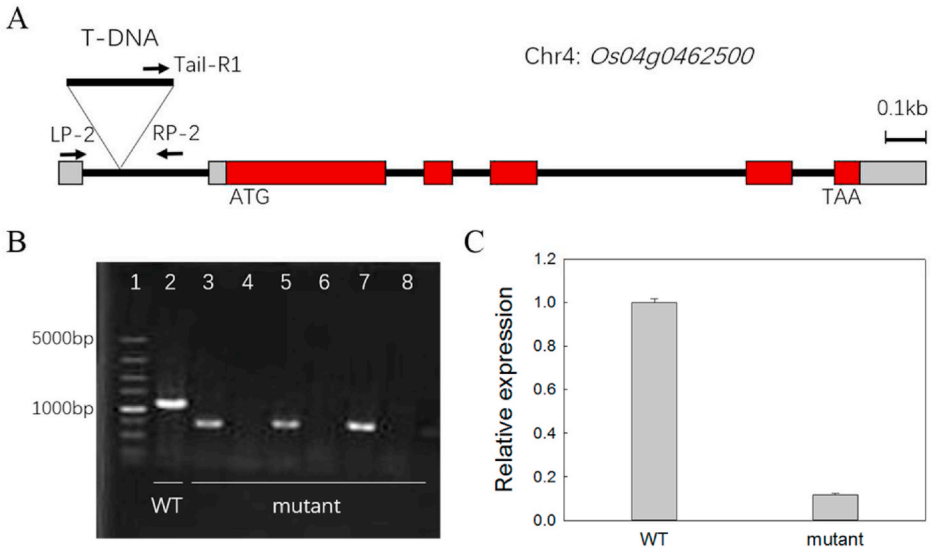

Fig. 1. T-DNA insertion location (A), Identification of insertion (B) and Relative expression of the *OsGF14b* gene in the WT and the mutant (C) (
B) Line 1: Marker; Line 2: the WT DNA was amplified by LP-2 and RP-2; Line 3, 5, 7: the mutant DNA was amplified by Tail-R1 and RP-2; Line 4, 6, 8: the mutant DNA was amplified by LP-2 and RP-2. Error bars indicate ± SD.

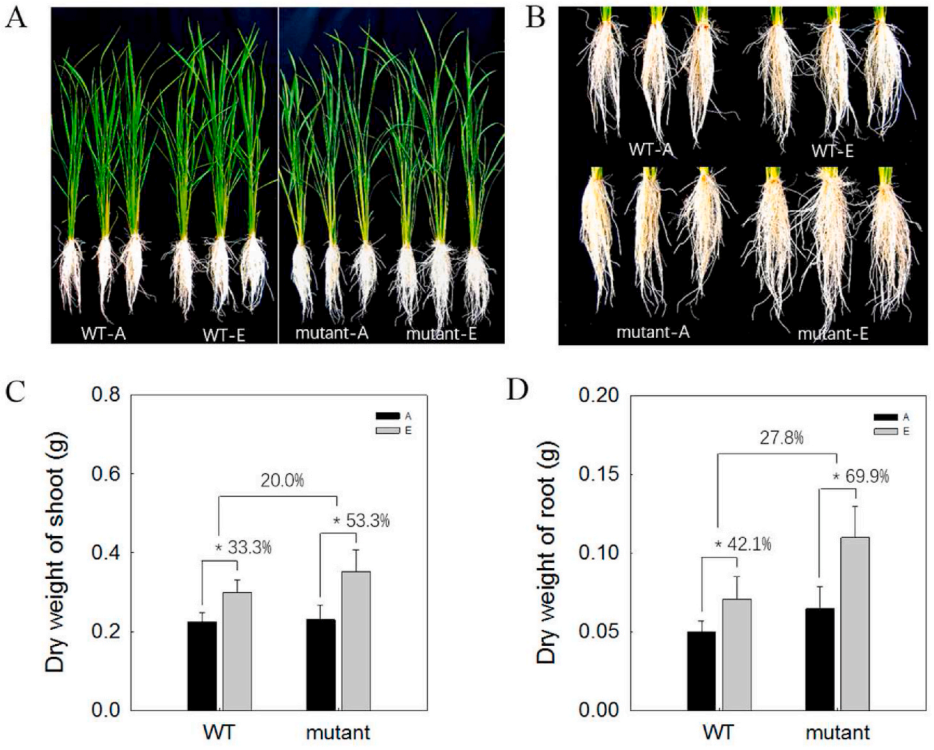

Fig. 3. Response to elevated [CO_2] of the WT and the mutant
WT-A: WT at control [CO_2]; WT-E: WT at elevated [CO_2]. Mutant-A: mutant at control [CO_2]; mutant-E: mutant at elevated [CO_2]. Asterisks indicate significant differences between two levels of CO_2 concentration. The percentages following asterisks are calculated as (E–A)/A. The percentages spanning between the two percentages indicate the differences between the latter and the former. *$P < 0.05$; bars are ± SD.

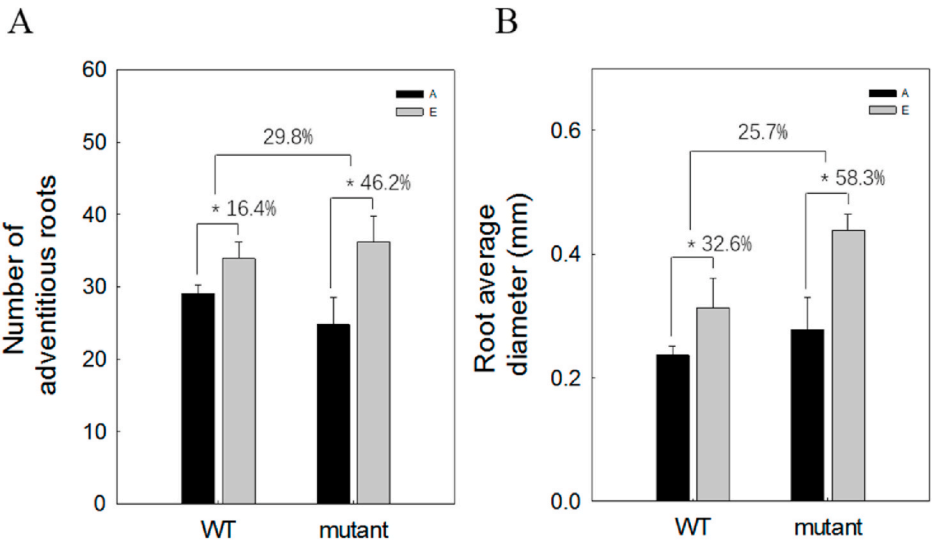

Fig. 4. Response of root architecture in the WT and the mutant at elevated [CO_2]
WT-A: WT at control [CO_2]; WT-E: WT at elevated [CO_2]. Mutant-A: mutant at control [CO_2]; mutant-E: mutant at elevated [CO_2]. Asterisks indicate significant differences between two levels of CO_2 concentration. The percentages are calculated as (E–A)/A. The percentages spanning between the two percentages indicate the differences between the latter and the former. *$P < 0.05$; bars are ± SD.

3.4. Root lengths in roots of different root diameters show different responses

Considering the characteristics of the fibrous root system in rice, the response of root lengths in roots of different diameters was further studied. Different responses to elevated [CO_2] were observed among root lengths of different diameters. In roots with a diameter of 0–0.2 mm, the mutant showed an increase of 23.2% in root length under elevated [CO_2], whereas no significant response was observed in the WT (Fig. 6A and S1). By contrast, the root lengths with diameters of 0.2–0.4 mm and 0.4–0.6 mm in the WT increased by 26.5% and 24.8% under elevated [CO_2], respectively, while the mutant showed no significant response (Fig. 6B and C). For the larger diameters of 0.6–0.8 mm and 0.8–1.0 mm, the responses of the mutant were significantly stronger than the WT under elevated [CO_2], with 33.8% in the WT and 56.7% in the mutant for the 0.6–0.8 mm diameter group, and 68.4% in the WT and 98.3% in the mutant for the 0.8–1.0 mm diameter group (Fig. 6D and E). The proportional breakdown of the root-length responses for the different diameter groups to elevated [CO_2] is shown in Fig. 7. Proportions in the mutant were significantly larger than WT in the 0–0.2 mm, 0.6–0.8 mm and 0.8–1.0 mm groups, while they were smaller in the 0.2–0.4 mm and 0.4–0.6 mm groups.

3.5. The strong response of the mutant under elevated [CO_2] was closely related to light intensity

Since light intensity is a key determinant to photosynthetic capacity in addition to [CO_2], a study at a weaker light intensity of 170 μmol m^{-2}

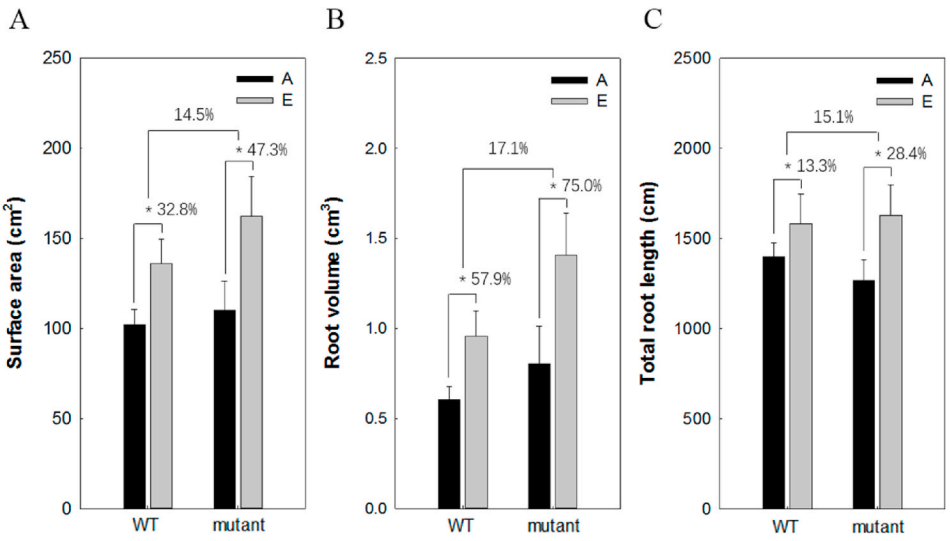

Fig. 5. Response of root surface area, root volume, and total root length in the WT and the mutant at elevated [CO_2]
WT-A: WT at control [CO_2]; WT-E: WT at elevated [CO_2]. Mutant-A: mutant at control [CO_2]; mutant-E: mutant at elevated [CO_2]. Asterisks indicate significant differences between two levels of CO_2 concentration. The percentages are calculated as (E–A)/A. The percentages spanning between the two percentages indicate the differences between the latter and the former. *P < 0.05; bars are ± SD.

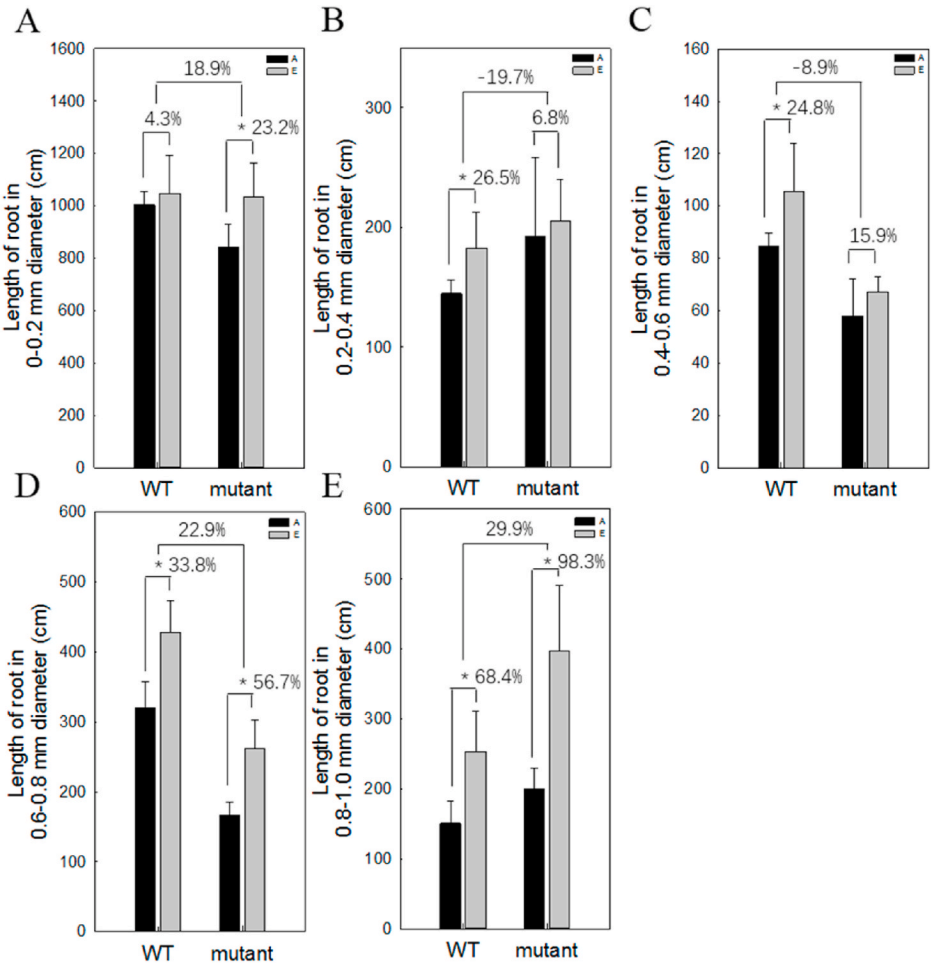

Fig. 6. Response of root length in roots of different diameter in the WT and the mutant at elevated [CO_2]
WT-A: WT at control [CO_2]; WT-E: WT at elevated [CO_2]. Mutant-A: mutant at control [CO_2]; mutant-E: mutant at elevated [CO_2]. Asterisks indicate significant differences between two levels of CO_2 concentration. The percentages are calculated as (E–A)/A. The percentages spanning between the two percentages indicate the differences between the latter and the former. *P < 0.05; bars are ± SD.

s^{-1} was also conducted. In contrast to the higher root and shoot response of the mutant than the WT to elevated [CO_2] seen at 1200 μmol m^{-2} s^{-1} (Fig. 3C and D), both shoot and root of the WT responded more pronouncedly than that of the mutant under elevated [CO_2] at 170 μmol m^{-2} s^{-1} (Fig. 8), showing that the response of the mutant under elevated [CO_2] is strongly related to light intensity.

4. Discussion

Elevated CO_2 concentrations in the atmosphere often increase photosynthetic rates and crop yields (Ainsworth et al., 2004; Kim et al., 2003; Long et al., 2004). In rice, yield responses under elevated [CO_2] vary substantially among cultivars, from 14% to over 30% (Ainsworth, 2008; Liu et al., 2008; Yang et al., 2006b; Zhu et al., 2013). To date,

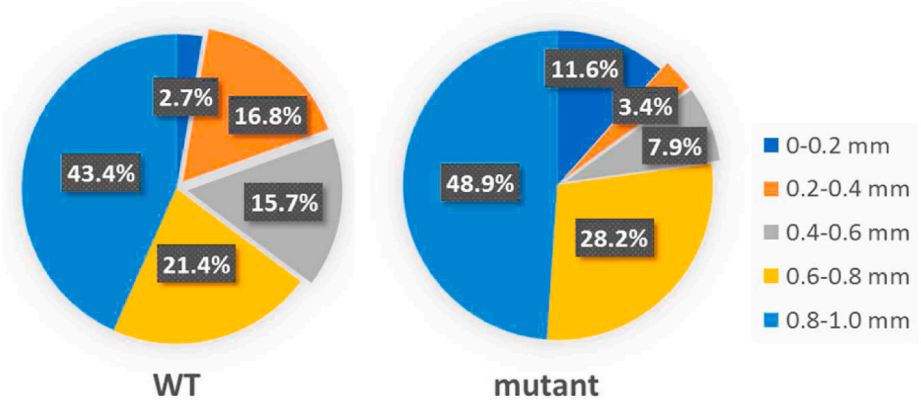

Fig. 7. The proportion of the root length response for roots of different diameter to elevated [CO_2] in relation to the total root length response.

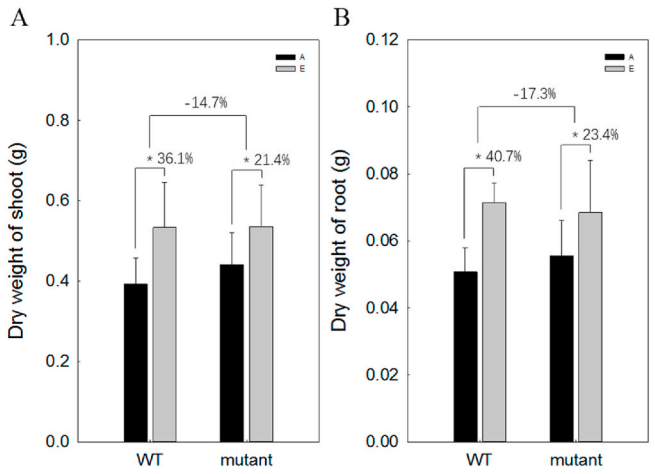

Fig. 8. The biomass response of the WT and the mutant to elevated [CO_2] under weak light exposure
WT-A: WT at control [CO_2]; WT-E: WT at elevated [CO_2]. Mutant-A: mutant at control [CO_2]; mutant-E: mutant at elevated [CO_2]. Asterisks indicate significant differences between two levels of CO_2 concentration. The percentages are calculated as (E–A)/A. The percentages spanning between the two percentages indicate the differences between the latter and the former. *P < 0.05; bars are ± SD.

however, the key genetic loci involved in the high-CO_2 response have not been identified. In this study, we have identified a genetic mutation that causes a significant change in the response to elevated [CO_2] in japonica rice. More than 20% enhancements were seen in both the aboveground and root biomass responses when ambient [CO_2] was raised from 380 to 760 ppm, with increases in root biomass reaching 27.8%. This change was similar to the yield differences among different rice varieties reported previously (Liu et al., 2008; Yang et al., 2006a; Zhang et al., 2015; Zhu et al., 2014), suggesting that the mutation site identified in our study might be associated with the response to elevated [CO_2] in rice more generally.

Root morphological analysis demonstrated that the number of adventitious roots, mean root diameter, root surface area, root volume, and the total root length of the rice mutant were all enhanced under elevated [CO_2] (Fig. 4 and 5), and these could explain why the root biomass response of the rice mutant was significantly greater than that of the WT (Fig. 3). Another interesting finding is that root diameters are related to CO_2 enhancement. The robust roots (>0.6 mm) and small lateral roots (<0.2 mm) of the mutant responded more significantly than in the WT under elevated [CO_2] (Fig. 6), which was correlated with the increase of root surface area and root volume (Fig. 5). It has been shown that the small lateral roots of rice can increase root biomass by absorbing more water and nutrients (Meng et al., 2019), while the robust roots are responsible for tolerance in harder-texture soils due to their strong bending stiffness (Jeong et al., 2013; Lynch, 2013). Given the strong response of mutant roots to elevated [CO_2], especially in the small lateral roots and coarse roots, deeper soil penetration and improved uptake of water and nutrients are enabled, thus increasing yield.

In addition to [CO_2], light intensity is a critical for photosynthtic capacity and, ultimately, yield in rice (Hubbart et al., 2012; Resurreccion et al., 2002). Many studies have focused on the genetic and physiological basis of light regulation, photoreceptors, and plant growth in response to light (Dutta et al., 2018; Jiao et al., 2005; Li et al., 2012; Petrillo et al., 2014). Hubbart et al. (2012) have shown that the photoprotective protein PsbS exerts control over the CO_2 assimilation rate under fluctuating light in rice. It has been shown that low light reduces the rate of overall growth and photosynthesis and impairs translocation of carbohydrates to developing grains, often resulting in sterility (Dutta et al., 2018). In our study, the superiority of the response to elevated [CO_2] in the mutant over the WT disappeared under weak light conditions (Fig. 8), indicating that the strength of the mutant response is a function of light intensity. Given that modern rice cultivars are bred and grown under high-light conditions, light may be the limiting factor for photosynthesis when CO_2 is saturated. Co-limitations with other key environmental factors, such as N supply, under elevated [CO_2], may also be expected (Coskun et al., 2016), and these relationships deserve future examination.

In this study, we found a T-DNA insertion mutant whose growth response in seedlings to elevated [CO_2] was significantly better than that of the WT, probably due to the change of an individual gene that led to the mutant's strong response. The insertion site was located in the promoter region of *OsGF14b*, resulting in a significant decrease in the expression of *OsGF14b* (Fig. 1C). Therefore, *OsGF14b* might act as a negative regulator of root architecture and rice growth under elevated [CO_2]. It was previously reported that *OsGF14b* played differential roles in panicle and leaf blast resistance (Liu et al., 2016), and functions in rice drought and osmotic resistance were also shown (Liu et al., 2019). However, this is the first report demonstrating that *OsGF14b* may be involved in the response of rice roots to elevated [CO_2]. Since the deletion mutant lacking *OsGF14b* showed a stronger response to elevated [CO_2] in the seedling stage, the inhibition of this gene's expression may carry potential for enhancing rice growth and, subsequently, yield under elevated [CO_2].

The negative regulation of root growth by *OsGF14b* under elevated [CO_2] may be related to the auxin transport and distribution in rice. Given that *OsGF14b* could induce the expression levels of the IAA synthesis-related proteins (Yan et al., 2021), auxin synthesis may be down-regulated when *OsGF14b* is defective. However, Li et al. (2011) showed that shoot-supplied ammonium inhibits lateral root primordium

emergence in *Arabidopsis* by interfering with auxin-influx-carrier-AUX1-dependent auxin transport from shoot to root, rather than with auxin content. Similar to the role of AUX1, it is reasonable to suggest that the knockout of *OsGF14b* in rice might optimize auxin transport from shoot to root, thereby promoting root growth. In addition, the regulation of *OsGF14b* may be dependent on leaf photosynthesis. Rubisco plays a central role in photosynthesis as well as in the N utilization of plants. However, the activity and regulation of Rubisco are not always optimal for photosynthesis and biomass production in various environments (Kanno et al., 2017). Under elevated [CO_2] conditions, previous studies showed that *rbcL* expression and Rubisco content in rice leaves was significantly decreased (Zhu et al., 2014). The greater yield response of the S63 rice cultivar under elevated [CO_2] was mainly linked to the maintenance of Rubisco content and gene expression (Zhu et al., 2014). Thus, given that the small Rubisco subunit is a target of the 14-3-3 protein (Schoonheim et al., 2007), it is possible that the absence of the 14-3-3 gene *OsGF14b* may alleviate the decline of Rubisco content and gene expression in rice leaves, thereby promoting rice growth. Although the exact function and mechanism of *OsGF14b* must be further studied, we have here provided a genetic basis for improved plant performance under CO_2 enhancement in a cultivar of the world's most important crop species.

In conclusion, we found the mutation of *OsGF14b* promotes biomass response to elevated [CO_2] in rice by over 20%, but this negative regulation of *OsGF14b* was not apparent under weak light. Under CO_2 enrichment, the knockout of *OsGF14b* accelerates the root response, especially the development of coarse roots and thin lateral roots, which is expected to promote the penetration of roots into denser soils and optimise the absorption of water and nutrients, improving the performance of the rice crop overall. As *OsGF14b* target the root system of rice, enhancing its growth and affecting its developmental program under elevated [CO_2], and given that more than 50% of plant biomass globally is belowground (Waisel et al., 1996; Rehling et al., 2021), the discovery offers important new insight into how enhanced belowground carbon storage may be achieved in plants.

Conclusion

In conclusion, we found the mutation of *OsGF14b* promotes biomass response to elevated [CO_2] in rice by over 20%, but this negative regulation of *OsGF14b* was not apparent under weak light. Under CO_2 enrichment, the knockout of *OsGF14b* accelerates the root response, especially the development of coarse roots and thin lateral roots, which is expected to promote the penetration of roots into denser soils and optimise the absorption of water and nutrients, improving the performance of the rice crop overall. As *OsGF14b* target the root system of rice, enhancing its growth and affecting its developmental program under elevated [CO_2], and given that more than 50% of plant biomass globally is belowground (Waisel et al., 1996; Rehling et al., 2021), the discovery offers important new insight into how enhanced belowground carbon storage may be achieved in plants.

Declaration of competing interest

The authors declare that there are no conflicts of interest.

CRediT authorship contribution statement

Jingjing Wu: Data curation, Formal analysis, Funding acquisition, Visualization, Writing – original draft. **Yufang Lu:** Funding acquisition, Resources, Writing – original draft. **Dongwei Di:** Formal analysis, Investigation, Methodology. **Yue Cai:** Funding acquisition, Resources. **Chuanhui Zhang:** Methodology, Validation. **Herbert J. Kronzucker:** Writing – review & editing. **Weiming Shi:** Conceptualization, Project administration, Writing – review & editing. **Kejun Gu:** Project administration, Supervision, Writing – review & editing, All authors discussed the results and commented on the manuscript. Each author certifies that this material or similar material has not been and will not be submitted to or published in any other publication before its appearance in the Journal of Plant Physiology.

Declaration of competing interest

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Acknowledgements

This work was supported by grants from the National Natural Science Foundation of China (32002124), the Key Research and Development Program of Jiangsu Province (BE2020319), the Natural Science Foundation for Excellent Young Scholar of Jiangsu Province of China (BK20190108), the Jiangsu Agricultural Independent Innovation Project (CX(19)1002), the Exploratory Disruptive Innovation Program of Jiangsu Academy of Agricultural Sciences (ZX(21)1202) and the University of Melbourne.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jplph.2021.153586.

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