Quantitative trait loci for large sink capacity enhance rice grain yield under free-air CO$_2$ enrichment conditions

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The global atmospheric CO$_2$ concentration has increased annually. To determine the trait that effectively increases rice (Oryza sativa L.) grain yield under increased atmospheric CO$_2$ concentrations, as predicted in the near future, we grew a chromosome segment substitution line (CSSL) and a near-isogenic line (NIL) producing high spikelet numbers per panicle (CSSL-GN1 and NIL-APO1, respectively) under free-air CO$_2$ enrichment (FACE) conditions and examined the effects of a large sink capacity on grain yield, its components, and growth-related traits under increased atmospheric CO$_2$ concentrations. Under ambient conditions, CSSL-GN1 and NIL-APO1 exhibited a similar grain yield to Koshihikari, as a result of the trade-off between increased spikelet number and reduced grain filling. However, under FACE conditions, CSSL-GN1 and NIL-APO1 had an equal or a higher grain yield than Koshihikari because of the higher number of spikelets and lower reduction in grain filling. Thus, the improvement of source activity by increased atmospheric CO$_2$ concentrations can lead to enhanced grain yield in rice lines that have a large sink capacity. Therefore, introducing alleles that increase sink capacity into conventional varieties represents a strategy that can be used to develop high-yielding varieties under increased atmospheric CO$_2$ concentrations, such as those predicted in the near future.

Crop growth and yield are affected by global changes in the environment, such as increasing atmospheric carbon dioxide (CO$_2$) concentrations and air temperature$^1$. The atmospheric CO$_2$ concentration has increased steadily from 280 $\mu$mol mol$^{-1}$ before the Industrial Revolution, to 400 $\mu$mol mol$^{-1}$ in 2015$^2$, and is projected to continue to increase over the course of this century.

The world's population is estimated to reach 9.1 billion people by 2050$^3$. To feed such a large number of people, global food production must be increased substantially. Rice (Oryza sativa L.) is eaten by nearly half of the world's population and is a staple food for most populations$^4$. Because arable land for rice is limited, improving rice yield per unit area is essential to resolve global food issues. However, the increased rice yield per unit area has been reduced in recent years throughout the world$^5$.

Terrestrial plants containing a C$_3$ photosynthetic pathway, including rice, are positively influenced by increased atmospheric CO$_2$ through photosynthetic rates and water-use efficiency$^6,7$. To produce high grain yield in rice, a large sink capacity is needed, as well as the ability to produce high levels of carbohydrates and to translocate them to the sink. Rice grain yield is thought to be improved in response to rising atmospheric CO$_2$ concentrations, as predicted in the near future. 

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concentrations through an increased number of spikelets per square meter\(^{8-12}\). High-yielding varieties with a large sink capacity (spikelet number per square meter \(\times\) single grain weight) have a higher grain-yield response to increased CO\(_2\) concentrations than conventional varieties with a general sink capacity\(^{11}\). Furthermore, there is a positive correlation between grain-yield response to increased CO\(_2\) concentrations and sink capacity. However, such high-yielding varieties with a large sink capacity generally possess other traits that allow them to produce a high grain yield. Therefore, direct evidence regarding the relationship between grain-yield response and increased CO\(_2\) concentration, and sink capacity is lacking.

Recent progress in rice genomics has facilitated genetic analyses of quantitative traits such as grain yield, and some genes regulating spikelet number per panicle of rice have been identified\(^{13,14}\). One quantitative trait loci (QTL) \(GN1a\), results in the production of more spikelets per panicle in the presence of an allele from an \(indica\) high-yielding variety than with that from a \(japonica\) conventional variety\(^{15}\). \(GN1a\) encodes the enzyme cytokinin oxidase/dehydrogenase (OsCXX2). In addition, \(GN1b\), which is estimated to lie in the vicinity of \(GN1a\), may also increase spikelet number per panicle with the allele from an indica high-yielding variety. Similarly, an allele of \(APO1\) derived from an \(indica\) high-yielding variety was found to produce a higher spikelet number per panicle than that from a \(japonica\) conventional variety\(^{16}\). However, in some field experiments performed under ambient conditions, near-isogenic lines (NIL) or chromosome segment substitution lines (CSSL) possessing such favorable alleles in the \(japonica\) genetic background did not have a higher grain yield due to the lower percentage of filled spikelets and 1000-grain weight\(^{15,16}\), suggesting a lack of source activity relative to their large sink capacity.

To date, several laboratory and chamber experiments investigating the plant growth response to increased atmospheric CO\(_2\) concentrations have been conducted and reported\(^{7}\). However, plant growth responses in the laboratory or under chamber conditions may differ from those observed under field conditions. Free-air CO\(_2\) enrichment (FACE) experiments represent a promising method to grow plants at controlled levels of elevated CO\(_2\) under fully open-air field conditions\(^{2}\) in order to investigate yield response to CO\(_2\) increases.

Increased atmospheric CO\(_2\) concentrations lead to an increase in the dry matter yield of rice\(^{19,20}\), indicating that source activity is raised. A previous rice FACE study showed that a variety Takanari with a large sink capacity is higher yielding and more responsive to increased CO\(_2\) concentrations than a conventional cultivar Koshihikari\(^{11}\). To raise yield potentials under increased CO\(_2\) concentrations efficiently and effectively, we need to understand the effects of QTLs that can enhance sink capacity on yield performance in increased CO\(_2\) under open field conditions. Recently, a CSSL and a NIL were developed carrying \(GN1\) and \(APO1\) alleles, respectively, from Takanari in the Koshihikari genetic background\(^{16}\). We therefore hypothesized that \(GN1\) and \(APO1\) alleles, which produce a higher spikelet number per panicle, have a high grain yield as a result of enhanced source activity under increased atmospheric CO\(_2\) concentrations. To test this hypothesis, we grew a conventional variety, Koshihikari, and CSSL-\(GN1\) and NIL-\(APO1\) with the Koshihikari genetic background under FACE conditions and examined the effects of a large sink capacity on grain yield, its components, and growth related traits under increased atmospheric CO\(_2\) concentrations (200\(\mu\)mol mol\(^{-1}\) above the ambient CO\(_2\)). On the basis of the results, we consider whether introducing such alleles to conventional varieties represents an effective method of increasing grain yield under increased atmospheric CO\(_2\) concentrations, such as those predicted in the near future.

**Results**

There were no significant interactions between year and CO\(_2\) concentration or among year, CO\(_2\) concentration, and genotype for grain yield, so data were combined over two years.

**Grain yield.** Grain yield was affected by CO\(_2\) concentration and genotype. There was an interaction between CO\(_2\) concentration and genotype for grain yield (Table 1). In Koshihikari, grain yield did not differ between CO\(_2\) concentrations (FACE/ambient = 1.08). However, in CSSL-\(GN1\) and NIL-\(APO1\), grain yield under FACE conditions was increased compared with under ambient conditions (FACE/ambient = 1.21 and 1.19, respectively). Under ambient conditions, CSSL-\(GN1\) and NIL-\(APO1\) had almost the same percentage and weight of filled spikelets and 1000-grain weight relative to Koshihikari, whereas NIL-\(APO1\) had almost the same percentage and weight as Koshihikari.

**Grain-yield components and harvest index.** Grain-yield components relating to spikelet number were affected by CO\(_2\) concentration and genotype (Table 1). In all tested genotypes, spikelet number per square meter, panicle number per square meter, and spikelet number per panicle did not differ between CO\(_2\) concentrations. Under both CO\(_2\) concentrations, CSSL-\(GN1\) and NIL-\(APO1\) had a higher single grain weight than Koshihikari, but had a much higher spikelet number per panicle. Consequently, CSSL-\(GN1\) and NIL-\(APO1\) had a higher spikelet number per square meter than Koshihikari.

Grain yield components relating to grain filling were influenced by CO\(_2\) concentration and genotype (Table 1). In all tested genotypes, the percentage of filled spikelets under FACE conditions was increased compared with that under ambient conditions. Under both CO\(_2\) concentrations, CSSL-\(GN1\) had low percentage of filled spikelets and 1000-grain weight relative to Koshihikari, whereas NIL-\(APO1\) had almost the same percentage and weight as Koshihikari.

There was an interaction between CO\(_2\) concentration and genotype for harvest index. Under ambient condition, CSSL-\(GN1\) had the lowest harvest index in all tested genotypes. However, under FACE condition, CSSL-\(GN1\) had a similar harvest index to Koshihikari. On the other hand, NIL-\(APO1\) had the highest harvest index in all tested genotypes consistently across two CO\(_2\) concentrations.

**Growth-related traits at heading.** The effects of CO\(_2\) concentration and genotype on growth-related traits at heading were examined (Table 2). In all tested genotypes, DM weight, stem DM weight, and NSC concentration under FACE conditions were increased compared with those under ambient conditions. As a result, the NSC
amount under FACE conditions was larger than that under ambient conditions. In addition, LAI did not differ between CO2 concentrations and among genotypes.

Growth-related traits at maturity. The effects of CO2 concentration and genotype on growth-related traits at maturity were examined (Table 3). In all tested genotypes, DM weight, stem DM weight, and NSC

| CO2 Concentration | Genotype | Grain Yield (g m⁻²) | Spikelet number (× 10⁶ m⁻²) | Panicle number (m⁻²) | Spikelet number (panicle⁻¹) | Percentage of filled spikelets (%) | 1000-grain weight (g) | Harvest Index |
|-------------------|----------|---------------------|-------------------------------|----------------------|----------------------------|-----------------------------------|------------------------|--------------|
| FACE              |          | 813                 | 47.0                          | 333                  | 143                        | 85.4                              | 20.5                   | 0.41         |
| Ambient           |          | 702                 | 44.4                          | 326                  | 138                        | 77.6                              | 20.6                   | 0.40         |

**Table 1.** Mean grain yield, its components, and harvest index as affected by different CO2 concentrations and genotypes averaged for two years (2012 and 2013). *Significant at P < 0.05. **Significant at P < 0.01. †Means within a column followed by the same lowercase letter do not differ significantly (P < 0.05). ‡Means within a column followed by the same lowercase letter do not differ significantly (P < 0.05) among genotypes for a given CO2 concentration. Means within a column followed by the same uppercase letter do not differ significantly (P < 0.05) between CO2 concentrations for a given genotype. §Significant at P < 0.10. ¶Not significant at P < 0.10.

| CO2 Concentration | Genotype | DM weight (g m⁻²) | Stem DM weight (g m⁻²) | NSC concentration (g kg⁻¹) | NSC amount (g m⁻²) | LAI |
|-------------------|----------|-------------------|------------------------|--------------------------|-------------------|-----|
| FACE              |          | 1152              | 713                    | 370                      | 264               | 4.33|
| Ambient           |          | 1038              | 609                    | 332                      | 203               | 4.67|

**Table 2.** Mean dry matter (DM) weight, nonstructural carbohydrate (NSC) concentration and its amount in the leaf sheaths plus stems, and leaf area index (LAI) at heading as affected by different CO2 concentrations and genotypes averaged for two years (2012 and 2013). *Significant at P < 0.05. **Significant at P < 0.01. †Not significant at P < 0.10.
**Significant at $P < 0.05$. §Significant at $P < 0.01$. †Dry matter increase from heading to maturity. **Significant at $P < 0.001$. ***Significant at $P < 0.0001$. **Significant at $P < 0.01$. *Significant at $P < 0.05$. ‡Means within a column followed by the same lowercase letter do not differ significantly ($P < 0.05$). ‡Means within a column followed by the same lowercase letter do not differ significantly ($P < 0.05$) among genotypes for a given CO$_2$ concentration. Means within a column followed by the same uppercase letter do not differ significantly ($P < 0.05$) between CO$_2$ concentrations for a given genotype. *Significant at $P < 0.10$. *Not significant at $P < 0.10$.  

### Table 3. Mean dry matter (DM) weight, nonstructural carbohydrate (NSC) concentration, and its amount in the leaf sheaths plus stems at maturity, and DM increase from heading to maturity ($\Delta W$) affected by different CO$_2$ concentrations and genotypes averaged for two years (2012 and 2013).

| CO$_2$ concentration | Genotype | DM weight (g m$^{-2}$) | Stem DM weight (g m$^{-2}$) | NSC concentration (g kg$^{-1}$) | NSC amount (g m$^{-2}$) | $\Delta W$ (g m$^{-2}$) |
|---------------------|----------|------------------------|-----------------------------|-------------------------------|------------------------|------------------------|
| FACE                |          | 1991                   | 786                         | 305                           | 241                    | 833                    |
| Ambient             |          | 1777                   | 664                         | 264                           | 176                    | 739                    |
| $C \times G$        |          |                        |                             |                               |                        |                       |
| FACE                | Koshihikari | 1874                   | 762a$^1$                   | 294                           | 227a$^1$               | 805                    |
| CSSL-GN1            |          | 1864                   | 691b                        | 286                           | 199b                   | 771                    |
| NIL-APO1            |          | 1913                   | 722ab                      | 273                           | 200b                   | 790                    |
| Ambient             | Koshihikari | 1811                   | 693                         | 262                           | 183b                   | 800                    |
| CSSL-GN1            |          | 1746                   | 644                         | 276                           | 179b                   | 711                    |
| NIL-APO1            |          | 1775                   | 654                         | 252                           | 166b                   | 707                    |
| ANOVA               |          |                        |                             |                               |                        |                       |
| CO$_2$ concentration | §        | *                      | **                          | **                            | NS                     |                       |
| Genotype (G)        | NS$^a$   | **                     | NS                          | *                             | NS                     |                       |
| $C \times G$        | NS       | NS                     | NS                          | NS                            | NS                     |                       |

### Table 4. Pearson correlation ($r$) analysis for agronomic traits in CSSL-GN1 and NIL-APO1 in 2012 and 2013.  

| Trait                  | Spikelet number ($\times 10^4$ m$^{-2}$) | Percentage of filled spikelets (%) | 1000-grain weight (g) | NSC amount at heading (g m$^{-2}$) | $\Delta W^*$ (g m$^{-2}$) |
|------------------------|-------------------------------------------|-----------------------------------|-----------------------|-----------------------------------|--------------------------|
| Grain yield            | $-0.046$                                  | $0.925^{***}$                     | $0.429$               | $0.874^{***}$                     | $0.610^*$                |
| Percentage of filled spikelets | $-0.375$                                  | $-$                               | $0.611^*$             | $0.762^{**}$                      | $0.574$                  |

### Concentration under FACE conditions were increased compared with those under ambient conditions. There was an interaction between CO$_2$ concentration and genotype for the NSC amount. In all tested genotypes, NSC amount was increased compared with that under ambient condition. Under ambient condition, the NSC amount did not differ among genotypes. However, under FACE condition, CSSL-GN1 and NIL-APO1 had a lower NSC amount than Koshihikari. In addition, $\Delta W$ did not differ between CO$_2$ concentrations and among genotypes.  

### Relationships between agronomic traits in CSSL-GN1 and NIL-APO1.  
Grain yield of CSSL-GN1 and NIL-APO1 was positively correlated with the percentage of filled spikelets, NSC amount at heading, and $\Delta W^*$ grain yield, but not with spikelet number per square meter and 1000-grain weight (Table 4). Also, the percentage of filled spikelets was positively correlated with correlated with 1000-grain weight and NSC amount at heading, but not with spikelet number per square meter and $\Delta W^*$.  

### Percentage of spikelet and spikelet weight at each position in the panicle.  
The percentage of spikelets at each position in the panicle was affected by genotype but not by CO$_2$ concentration (Fig. 1 and Table 5). Under both CO$_2$ concentrations, CSSL-GN1 and NIL-APO1 had a lower percentage of primary and secondary spikelets than Koshihikari, but a higher percentage of tertiary spikelets.  
Spikelet weight at each position in the panicle was influenced by CO$_2$ concentration and genotype (Fig. 1 and Table 5). There was an interaction between CO$_2$ concentration and genotype for primary spikelet weight. In Koshihikari, the primary spikelet weight under FACE condition was increased compared with that under ambient condition. However, in CSSL-GN1 and NIL-APO1, the primary spikelet weight did not differ between CO$_2$ concentrations. Under ambient conditions, primary spikelet weight did not differ among genotypes. However, under FACE conditions, CSSL-GN1 had a lower primary spikelet weight relative to Koshihikari, whereas NIL-APO1 had
a similar primary spikelet weight to Koshihikari. In all tested genotypes, the tertiary spikelet weight under FACE conditions was increased compared with that in ambient. Under both CO2 concentrations, CSSL-GN1 had low secondary and tertiary spikelet weights relative to Koshihikari, whereas NIL-APO1 had almost the same secondary and tertiary spikelet weights as Koshihikari.

**Spikelet number and weight per square meter at each position in the panicle.** Spikelet number at each position in the panicle per square meter were affected by CO2 concentration and genotype (Fig. 1 and Table 6). In all tested genotypes, the tertiary spikelet number per square meter under FACE conditions was increased compared with that under ambient conditions. Under both CO2 concentrations, CSSL-GN1 had a higher secondary spikelet number than Koshihikari, whereas NIL-APO1 had almost the same number as Koshihikari. Similarly, CSSL-GN1 had the highest tertiary spikelet number, followed by NIL-APO1 and Koshihikari.

Spikelet weight at each position in the panicle per square meter was influenced by CO2 concentration, but not by genotype (Fig. 1 and Table 6). In all genotypes, tertiary spikelet weight under FACE conditions was increased compared with that under ambient conditions.

**Discussion**

The results of previous studies have indicated that CSSL and NIL, carrying GN1 or APO1 alleles derived from high-yielding indica varieties, did not exhibit high grain yield despite their large sink capacity\(^{15,16}\). In the present study, CSSL-GN1 and NIL-APO1 had almost the same grain yield as Koshihikari under ambient conditions because of the trade-off between increased spikelet number and reduced grain filling (Table 1). Furthermore, CSSL-GN1 had a lower grain yield than NIL-APO1. The low grain yield of CSSL-GN1 may result from its extremely large sink capacity. These results confirmed that high source activity is required to increase grain yield in rice lines that have a large sink capacity.

In contrast to this, under FACE conditions, CSSL-GN1 and NIL-APO1 had an equal or a higher grain yield than Koshihikari because of the higher number of spikelets and lower reduction in grain filling. In addition,
although the effect of increased atmospheric CO2 concentration on spikelet number was not clear in the present study (Table 1), spikelet number per square meter was reportedly increased by increased atmospheric CO2 concentrations8–12, 18.

Grain carbohydrates are derived from both accumulated carbohydrates (i.e., NSC) in the leaf sheaths plus stems at heading and photosynthetic products during grain filling (i.e., ΔW)19. The contributions of NSC at heading and ΔW to grain carbohydrates range from 0 to 40% and 60 to 100%, respectively, under most weather conditions during grain filling. However, NSC at heading is considered to be important to stabilize grain yield under unfavorable weather conditions during grain filling, because it compensates for the reduction in ΔW20–22. The NSC amount at heading in the present study were increased by increased atmospheric CO2 concentrations in all tested genotypes (Tables 2). The large amount of NSC at heading resulted from the stem DM weight, and growth rate (CGR) during the late reproductive period23. The high CGR led to the production of a high number of spikelets per square meter and a high level of NSC amount at heading, which was associated with the rapid translocation of NSC to the panicle during the initial period of grain filling. In the present study, the grain yield of CSSL-GN1 and NIL-APO1 was related to the percentage of filled spikelets and NSC amount at heading, and the percentage of filled spikelets was related to the NSC amount at heading (Table 4). In addition, the results of a recent study revealed that spikelet sterility of varieties with a high number of spikelets was caused by a lack of assimilate supply around flowering24. Thus, improved source activity in response to increased atmospheric CO2 concentration can enhance grain yield in rice lines that have a large sink capacity.

In the present study, in all tested genotypes, NSC amount at maturity was increased by increased atmospheric CO2 concentrations4–12, 18.

### Table 5. Mean percentage of spikelet and spikelet weight per spikelet at each position (primary, secondary, tertiary, and quaternary) in the panicles as affected by different CO2 concentrations and genotype averaged for two years (2012 and 2013). *Significant at P < 0.05. **Significant at P < 0.01. §Means within a column followed by the same lowercase letter do not differ significantly (P < 0.05) among genotypes for a given CO2 concentration. Means within a column followed by the same lowercase letter do not differ significantly (P < 0.05) between CO2 concentrations for a given genotype. †Significant at P < 0.10. ‡Not significant at P < 0.10.

| CO2 concentration | Genotype | Percentage of spikelet | Spikelet weight |
|-------------------|----------|------------------------|----------------|
|                   |          | Primary (%) | Secondary (%) | Tertiary (%) | Quaternary (%) | Primary (mg spikelet−1) | Secondary (mg spikelet−1) | Tertiary (mg spikelet−1) | Quaternary (mg spikelet−1) |
| FACE (C)          |          | 6.7         | 52.8         | 40.4         | 0.0           | 24.6               | 23.6               | 18.7               | 0.3                   |
| Ambient           |          | 6.9         | 53.6         | 39.6         | 0.0           | 23.9               | 23.3               | 15.5               | 0.0                   |
| Genotype (G)      |          |            |              |              |              |                    |                    |                    |                      |
| Koshihikari       |          | 7.7a      | 57.5a        | 34.8b       | 0.0          | 24.7a              | 24.5a              | 19.2a              | 0.0                   |
| CSSL-GN1          |          | 6.2b      | 50.5b        | 43.3a       | 0.0          | 23.6b              | 21.9b              | 14.3b              | 0.0                   |
| NIL-APO1          |          | 6.6b      | 51.5b        | 41.9a       | 0.1          | 24.5a              | 24.0a              | 17.8a              | 0.5                   |
| C × G             |          |            |              |              |              |                    |                    |                    |                      |
| FACE Koshihikari  |          | 7.7       | 57.6         | 34.7        | 0.0          | 25.6aA          | 25.1               | 21.5               | 0.0                   |
| CSSL-GN1          |          | 6.0       | 49.8         | 44.2        | 0.0          | 23.3b              | 21.8               | 15.1               | 0.0                   |
| NIL-APO1          |          | 6.5       | 51.0         | 42.5        | 0.1          | 24.8a              | 24.0               | 19.4               | 1.0                   |
| Ambient           |          | 7.7       | 57.4         | 34.8        | 0.0          | 23.8b              | 23.9               | 16.9               | 0.0                   |
| CSSL-GN1          |          | 6.3       | 51.3         | 42.5        | 0.0          | 23.9               | 22.1               | 13.4               | 0.0                   |
| NIL-APO1          |          | 6.7       | 52.1         | 41.3        | 0.0          | 24.1               | 24.1               | 16.1               | 0.0                   |
| ANOVA             |          |            |              |              |              |                    |                    |                    |                      |
| CO2 concentration (C) | NS‡       | NS         | NS           | NS          | NS          | NS                 | NS                 | NS                 | NS                   |
| Genotype (G)      |          | **        | **           | **          | NS §       | *                 | +                  | NS                 | NS                   |
| C × G             |          | NS         | NS           | NS          | NS         | §                  | NS                 | NS                 | NS                   |

*Significant at P < 0.05. **Significant at P < 0.01. §Means within a column followed by the same lowercase letter do not differ significantly (P < 0.05) among genotypes for a given CO2 concentration. Means within a column followed by the same lowercase letter do not differ significantly (P < 0.05) between CO2 concentrations for a given genotype. †Significant at P < 0.10. ‡Not significant at P < 0.10.
Table 6. Mean spikelet number and spikelet weight per square meter at each position (primary, secondary, tertiary, and quaternary) in the panicles as affected by different CO2 concentrations and genotypes averaged for two years (2012 and 2013). *Significant at P < 0.05. **Significant at P < 0.01. 1Means within a column followed by the same lowercase letter do not differ significantly (P < 0.05). 2Significant at P < 0.10. §Not significant at P < 0.10.

| CO2 concentration (C) | Spikelet number | Spikelet weight |
|-----------------------|-----------------|-----------------|
|                       | Primary (×10^3 m^-2) | Secondary (×10^3 m^-2) | Tertiary (×10^3 m^-2) | Quaternary (×10^3 m^-2) | Primary (g m^-2) | Secondary (g m^-2) | Tertiary (g m^-2) | Quaternary (g m^-2) |
| FACE                  | 3.1             | 24.6            | 19.2            | 0.0                  | 77              | 579            | 350              | 0.0                  |
| Ambient              | 3.0             | 23.7            | 17.7            | 0.0                  | 72              | 552            | 271              | 0.0                  |
| Genotype (G)         |                 |                 |                 |                      |                 |                 |                 |                      |
| Koshihikari          | 3.1             | 23.3b          | 14.1c           | 0.0                  | 77              | 569            | 271              | 0.0                  |
| CSSL-GN1             | 3.2             | 26.0a          | 22.2a           | 0.0                  | 74              | 570            | 319              | 0.0                  |
| NIL-APO1             | 3.0             | 23.2b          | 19.0b           | 0.0                  | 72              | 558            | 341              | 0.0                  |
| Anova                |                 |                 |                 |                      |                 |                 |                 |                      |
| CO2 concentration (C) | NS§             | NS              | NS              | NS                  | NS              | NS              | NS               | ‡                    |
| Genotype (G)         | §               | *               | **              | NS                  | §               | NS              | NS               | NS                  |
| C × G                | NS              | NS              | NS              | NS                  | NS              | NS              | NS               | NS                  |

and NIL-APO1, increased atmospheric CO2 concentrations may increase the tertiary grain weight per spikelet in spite of their large tertiary spikelet number per square meter resulting in an increased percentage of filled spikelets. In a recent study, Koshihikari and Takanari were compared, and Takanari, which has a higher number of spikelets, showed a substantial increase in grain weight of inferior spikelets under FACE conditions, while the weight of superior spikelets was not affected under those conditions. The results of the present study confirm that the increased weight of the inferior spikelets could be enhanced by increased source capacity, and could contribute to a greater yield response to elevated CO2 conditions. It is worth noting, however, that the source capacity for Takanari was also significantly greater than that for Koshihikari under both current and future CO2 conditions, suggesting that an increase in source capacity is also needed to meet the increased demand of grain for carbon.

To resolve global food issues, there is a need to develop high-yielding varieties that can adjust to the future environment. Recently developed high-yielding varieties in Japan are divided into two groups, inbred varieties from indica and indica-japonica varieties (i.e., pureline varieties originated from the cross of indica and japonica parents). Indica high-yielding varieties developed in Japan have higher percentage of filled spikelets than indica-japonica high-yielding varieties, because they have higher grain weight of tertiary spikelet. This suggests that they are able to produce high levels of carbohydrates and translocate them to their sink. Indica-japonica high-yielding varieties have a higher sink capacity than indica high-yielding varieties developed in Japan. Thus, such varieties are expected to produce a high grain yield under the increased atmospheric CO2 concentrations that may occur in the near future.

Lodging reduces grain yield as a result of self-shading and reduced canopy photosynthesis. The results of a previous report indicated that lodging was increased by increasing panicle weight under ambient conditions, but was alleviated under FACE conditions. Consequently, to develop varieties which can contribute a stable and high production of rice under increased atmospheric CO2 concentrations in the near future, introducing alleles to conventional varieties that enhance sink capacity represents a useful strategy, because the risk of lodging as a results of increasing panicle weight is relatively low.

Materials and Methods

Experimental design and crop management. The study was conducted in 2012 and 2013 on a Fluvisol, which is typical of alluvial areas, at the Tsukubamirai free-air CO2 enrichment field (35°58’N, 139°60’E, 10 m above sea level), Tsukubamirai, Ibaraki, Japan. Rice was grown previously in the field was rice in both years. Treatments included two atmospheric CO2 concentrations and three genotypes (one variety and two lines), which were arranged as a split-plot experiment with four replicates in a randomized complete block design. The main plot and subplot were atmospheric CO2 concentration and genotype, respectively. The mean temperature and...
Grains were counted with a multi auto counter (KC-10, Fujiwara Seisakusho, Tokyo, Japan) to determine the spikelet 1000-grain weight were corrected based on a 150 g kg⁻¹ moisture concentration. Approximately 100 g of rough rice grains were counted with a multi auto counter (KC-10, Fujisawa Seisakusho, Tokyo, Japan) to determine the spikelet 1000-grain weight. Grain yield and 1000-grain weight were calculated from this value. Grain yield and 1000-grain weight were corrected based on a 150 g kg⁻¹ moisture concentration. Approximately 100 g of rough rice grains were counted with a multi auto counter (KC-10, Fujisawa Seisakusho, Tokyo, Japan) to determine the spikelet 1000-grain weight.

Grain numbers with a thickness of 1.6 mm or more making up 20 g were counted with a multi auto counter (KC-10, Fujiwara Seisakusho, Tokyo, Japan). The 1000-grain weights were calculated from this value. Grain yield and 1000-grain weight were corrected based on a 150 g kg⁻¹ moisture concentration. Approximately 100 g of rough rice grains were counted with a multi auto counter (KC-10, Fujisawa Seisakusho, Tokyo, Japan) to determine the spikelet 1000-grain weight.

Grain yield and 1000-grain weight were corrected based on a 150 g kg⁻¹ moisture concentration. Approximately 100 g of rough rice grains were counted with a multi auto counter (KC-10, Fujisawa Seisakusho, Tokyo, Japan) to determine the spikelet 1000-grain weight.

Table 7. Mean temperature and solar radiation at the Tsukubamirai free-air CO₂ enrichment (FACE) facility, Tsukubamirai, Ibaraki, Japan, during the 2012 and 2013 crop seasons. †30-yr average (1981–2010) recorded at the nearest weather station Tateno.

| Month  | Stage of month | Mean temperature  | Solar radiation |
|--------|----------------|-------------------|----------------|
|        |                | 2012 (°C)         | 2013 (°C)       | Normal¹ (°C) | 2012 (MJ m⁻² d⁻¹) | 2013 (MJ m⁻² d⁻¹) | Normal (MJ m⁻² d⁻¹) |
| May    | early          | 19.2              | 20.1            | 17.9         | 21.4  | 20.1  | 21.4  |
|        | middle         |                   |                 |              |       |       |       |
|        | late           |                   |                 |              |       |       |       |
| June   | early          | 19.9              | 20.1            | 19.4         | 18.5  | 22.7  | 17.8  |
|        | middle         |                   |                 |              |       |       |       |
|        | late           |                   |                 |              |       |       |       |
| July   | early          | 22.9              | 25.6            | 22.5         | 17.6  | 20.0  | 15.2  |
|        | middle         |                   |                 |              |       |       |       |
|        | late           |                   |                 |              |       |       |       |
| August | early          | 26.2              | 26.6            | 25.8         | 20.5  | 19.2  | 18.1  |
|        | middle         |                   |                 |              |       |       |       |
|        | late           |                   |                 |              |       |       |       |
| September | early     | 25.4              | 25.0            | 24.0         | 16.1  | 13.9  | 14.4  |
|        | middle         |                   |                 |              |       |       |       |
|        | late           |                   |                 |              |       |       |       |

CO₂ control. The atmospheric CO₂ concentration was controlled as described by Nakamura et al. The average atmospheric CO₂ concentration ± day-to-day standard deviations during the crop season in FACE plots was 578 ± 15.7 μmol mol⁻¹ in 2012 and 576 ± 15.5 μmol mol⁻¹ in 2013, and in ambient plots was 383 ± 11.2 μmol mol⁻¹ in 2012 and 383 ± 11.4 μmol mol⁻¹ in 2013.

Sampling and measurements. At heading (Zadoks code 59) (early August), plants from 0.405 m² (nine hills) were sampled. Two hills with an average panicle number were selected and separated into green leaf blades, dead leaf blades, leaf sheaths plus stems, and panicles. After the area of green leaf blades was measured with a leaf area meter (AAM-9, Hayashi Denko, Tokyo, Japan), each plant part was dried at 80 °C in a ventilated oven for 2 days with the plants of the remaining hills to determine their dry weight. The dried samples were ground to a powder with a vibrating sample mill (TI-1001, CMT, Co., Ltd., Tokyo, Japan) in order to measure nonstructural carbohydrate (NSC) concentrations. Concentrations of NSCs in the leaf sheaths plus stems were determined as described by Ohnishi and Horie.

At maturity (Zadoks code 92) (mid-September), plants from 0.810 and 0.855 m² (18 and 19 hills) in 2012 and 2013, respectively, were sampled. Two hills were selected with an average panicle number and were separated into leaf blades, leaf sheaths plus stems, and panicles. Their NSC concentrations were determined as described. The 15 hills were air-dried until they reached a constant weight. The panicles were counted, and the air-dried plants were threshed. Half of the rough rice grains were dehusked to determine the grain weight and the 1000-grain weight. Grain yields and 1000-grain weight were corrected based on a 150 g kg⁻¹ moisture concentration.
numbers. The remaining two hills were harvested and air-dried until they reached a constant weight. The spikelet numbers at each position (primary, secondary, tertiary, and quaternary) in the three panicles with greater weights were recorded as described in Fig. 1, and then the weights of the spikelets were determined.

**Statistical analysis.** Statistical analyses were performed using a general linear model in SPSS (SPSS Inc., Chicago, IL, USA). CO₂ concentration and genotype were considered as fixed effects. Year and replication were considered as random effects. Analysis of variance (ANOVA) was conducted to test the effects of CO₂ concentration and genotype on yield, its components, and panicle structure. Significant treatment effects (P < 0.05) were explored using Fisher's protected least significant difference (LSD).

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Author Contributions
H.N., S.Y., T.H. and M.K. designed the experiment. H.N., S.Y., T.T., Y.A.-S., H.S., T.T., Y.U., H.N., T.H. and M.K. performed the experiment. K.K. and T.Y. developed NIL-APO1. H.N. analyzed the results. H.N., T.H. and M.K. wrote the manuscript. S.Y., T.T., Y.A.-S., K.K., T.Y., H.S., T.T., Y.U. and H.N. provided advise on the manuscript.

Additional Information
Competing Interests: The authors declare that they have no competing interests.

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