Varietal difference in dynamics of non-structural carbohydrates in nodal segments of stem in two varieties of rice (*Oryza sativa* L.) at pre- and post-heading stages

Yu Wakabayashi, Ryutaro Morita, Junko Yamagishi and Naohiro Aoki

Graduate School of Agricultural and Life Sciences, The University of Tokyo, Tokyo, Japan

ABSTRACT

The non-structural carbohydrate (NSC) stored in rice stems (culm plus leaf sheaths) at pre-heading stage is one of the major carbon sources for grain filling. In this study, we conducted field experiments in 2018 and 2019 using two varieties, Momiroman (japonica-dominant) and Teqing (indica), which have different accumulation and remobilization patterns of NSC in the stems. We analyzed NSC dynamics of each of the five nodal segments of internodes and leaf sheaths at pre- and post-heading stages. The dynamics of NSC at pre- and post-heading stages differed among segments. The lower segments of internodes and leaf sheaths accumulated more NSC at pre-heading stage compared to the upper segments in both varieties. The lower internodes in Teqing were morphologically larger than in Momiroman and would be able to store more NSC. However, the upper parts of the stems required biomass for development and formation at the stage of NSC accumulation in stems. Momiroman had longer upper internodes and leaf sheaths and required more biomass than Teqing. The NSC remobilization was later in the upper than the lower internodes for both varieties, which led to the delay of NSC remobilization in the entire culm after heading. These results indicated that ideal morphology of stems, to maximize the potential contribution of stem NSC as the carbon source for grain filling, is large lower internodes and short upper internodes and leaf sheaths.

ARTICLE HISTORY

Received 8 February 2021
Revised 12 March 2021
Accepted 4 April 2021

KEYWORDS

Rice (*Oryza sativa* L.); non-structural carbohydrates; assimilate partitioning; internode elongation

Introduction

Rice (*Oryza sativa* L.) is one of the most important staple food crops for approximately 50% of the world’s population, and supplies approximately 19% of the total calories consumed by the entire human population (FAO, 2017; IRRI, 2016). Due to the growing population and changing climate, with little scope to expand arable land, yield potential and yield stability need to be enhanced (Khush, 2005; Powell et al., 2012).

CONTACT Naohiro Aoki

aaokin@g.ecc.u-tokyo.ac.jp

Supplemental data for this article can be accessed here.

© 2021 The Author(s). Published by Informa UK Limited, trading as Taylor & Francis Group.

This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.
The Carbon (C) sources for grain development consist of two components: non-structural carbohydrate (NSC) accumulated in stems at the pre-heading stage and newly assimilated NSC at post-heading stages. Approximately 30% and 70% of the final NSC content in grains is derived from the former and the latter respectively, making post-heading the main C source for grain filling (Cock & Yoshida, 1972). However, the pre-heading contribution of NSC to grain filling increases under adverse climatic conditions such as low radiation (Nagata et al., 2001; Okamura et al., 2013; Ookawa et al., 2003), water deficit (Yang et al., 2001) and high temperature (Morita & Nakano, 2011), and has a buffering effect against yield loss. It is expected that a larger maximum amount of NSC stored in stems will enhance this potential buffering effect. In not only adverse but also normal climatic conditions, NSC stored until heading plays a role in the improvement of grain-filling traits (Fu et al., 2011; Ishimaru, 2003; Ishimaru et al., 2005).

It was reported that the capacity to accumulate NSC in stems depended on the rice variety, and indica or indica-dominant varieties tended to store more NSC than japonica varieties in stems at the heading stage (Ishikawa et al., 1993; Samonte et al., 2001). Differences in NSC translocation patterns were also reported among the rice subspecies. The indica-dominant varieties exhibit earlier translocation of stem NSC to panicles after heading compared with japonica-dominant varieties and this trait contributes to the grain-filling ability during the early ripening period (Wada et al., 2017; Yoshinaga et al., 2013). Although, as mentioned above, there are differences among subspecies in the capacity for NSC accumulation and the timing of NSC remobilization in stems, the factors related to these differences in NSC dynamics in stems at pre- and post-heading stages are not fully understood.

There is a difference in NSC dynamics among components of the stem, namely the leaf sheath and culm. Some reports indicate that NSC of the leaf sheath is remobilized earlier than that of the culm (Nagata et al., 2001; Okamura et al., 2018). Furthermore, in the leaf sheath, NSC dynamics also differ depending on position, with the second leaf sheath below the flag leaf accumulating much more starch than leaf sheaths at other positions (Ishimaru et al., 2004; Watanabe et al., 1997). The culm is a sink organ that elongates as well as accumulating NSC during the pre-heading stage. It is evident that the upper internodes of the culm undergo rapid elongation at the NSC-accumulating stage in stems. Fujita and Yoshida (1984) indicated that dry matter partitioning and starch synthesis differ among varieties with different lengths of culm and leaf sheaths, and suggested that the elongating culm is a strong competitor for assimilates with other vegetative organs and the panicle just before heading stage. However, the differences in NSC accumulation pattern and dry matter partitioning among positions of the culm and leaf sheath at pre- and post-heading stages remain unclear. The objective of this study is to elucidate the NSC dynamics occurring in each position in stems at pre- and post-heading stages and the factors related to varietal differences in the traits of NSC accumulation and remobilization in stems. For this purpose, we used two rice varieties, Momiroman and Teqing. Momiroman, released in 2008, is a japonica-dominant variety developed in Japan (Yamamoto et al., 2010) and shows later NSC remobilization from stems and a lower grain-filling rate compared to indica-dominant varieties (Wada et al., 2017; Yoshinaga et al., 2013). Teqing, released in 1984, is an indica variety developed in China (Wu et al., 1998) and stores a larger amount of NSC in stems at heading and translocates it earlier to panicles after heading compared with Momiroman (Okamura et al., 2018). Using analysis of NSC dynamics of nodal segments in these two varieties, we propose the ideal morphological traits of stems in order to maximize the contribution of NSC stored in stems to grain filling.

Material and methods

Field experimental details

Field experiments were conducted in 2018 and 2019 in the paddy field at the Institute for Sustainable Agro-ecosystem Services (ISAS), University of Tokyo (35°44′N, 139°32′E, altitude: 58 m), Tokyo, Japan. Seeds of Momiroman and Teqing (provided from the National Agriculture and Food Research Organization, Tsukuba, Japan) were sown in a seedling nursery box, and seedlings were transplanted at about three weeks old on 30 May in 2018 and 28 May in 2019, with three seedlings per hill. The planting density was 22.2 hills m⁻² with 30-cm row spacing and 15-cm intra row spacing. Compound fertilizer was applied at 6 g N m⁻², 8 g P₂O₅ m⁻², and 9 g K₂O m⁻² as a basal dressing. No top-dressing of N fertilizer was applied, expecting to see clear varietal differences in NSC dynamics, because it has been reported that low N conditions accelerate the accumulation and remobilization of NSC in stems at pre-and post-heading stages (G. Li et al., 2018; Pan et al., 2011). Weeds, insects and diseases were prevented by applying herbicides and pesticides as needed. The plots were arranged in a randomized block design with three replicates, and each plot was 24 m².
Measurement of dry matter production and NSC contents

For each plot of three replicates, main stems of four plants per plot were sampled 7–8 times from the young panicle formation date to about 30 days after the heading date (DAH). The young panicle formation date was defined as the date when its length reached 2 mm, and the heading date of the main stem was defined as the date when the top part of the panicle emerged from the leaf sheath of the flag leaf. Internodes and leaf sheaths of main stems were separated according to the following definition. The uppermost internode and leaf sheath attached to the flag leaf were defined as the 1st internode and leaf sheath, respectively, and the segments below it were numbered in order from 2nd. Internodes and leaf sheaths from the 1st to the 5th position were separately sampled because both varieties had five elongated internodes in both years. The sampling of the 1st and 2nd leaf sheaths was conducted from the time when the leaf blades attached to them were completely emerged from the next leaf sheath below. The length of each internode and leaf sheath was measured at all sampling times. To assess the diameter of each internode, the outer diameter of the central position of each internode was measured at the middle of the grain-filling period. Each internode and leaf sheath was weighed after drying for at least 72 h at 80 °C. For each part of the leaf sheaths and internodes, the dried segments of four individuals were combined into one sample and ground to powder using the Multi-Beads Shocker (Yasui Kikai, Osaka, Japan) for NSC measurements. The dry weight of panicles attached to main stem was also determined after heading using the same drying conditions.

The contents of starch and soluble sugar (sucrose, glucose and fructose) were measured according to Okamura et al. (2013) with glucoamylase (Toyobo, Osaka, Japan), F-kit #716,260 (J.K. International, Tokyo, Japan) and a microplate reader (Viento XS, DS Pharma Biomedical, Osaka, Japan).

Statistical analysis

Analysis of variance was performed using ‘STAR (Statistical Tool for Agricultural Research)’ software, IRRI, according to the randomized complete block design to assess varietal differences and the effects of year and of variety × year interaction. Differences in mean values were determined using Tukey’s test (P < 0.05).

Results

Phenological events and climatic conditions

The young panicle formation and heading dates were similar for the two varieties in both years (Supplemental Table S1). At the pre- and post-heading stages, solar radiation conditions differed between the two years (Supplemental Figure S1). The solar radiation from 20 to 10 days before the heading date (DBH) was lower in 2018 than in 2019. However, in the following 18 days, solar radiation was higher in 2018 than in 2019. During 10 to 25 DAH, solar radiation was similar but, in the later grain-filling period, was lower in 2018 than in 2019. The temperature was higher in 2018 than in 2019 during about one week after heading, while it was higher in 2019 than in 2018 during 30 to 40 DAH.

Morphological traits of main stem

The internode elongation occurred in order from lower to upper internodes (Figure 1). The 4th and 5th internodes had already elongated to some degree by young panicle formation in both years. The elongation of the 3rd internode occurred during the period from about 20 to 8 DBH in Momiroman, but continued until heading stage in Teqing. Elongation of the 1st and 2nd internodes started from about 5 and 10 DBH, respectively, and was completed at about 5 DAH. In the 4th and 5th internodes, the final lengths were greater in Teqing than in Momiroman, but the final lengths of 1st and 2nd internodes were less in Teqing than Momiroman in both years (Table 1). The culm diameter of the 4th and 5th internodes was larger in Teqing than in Momiroman in both years. The other segments except for the 5th leaf sheath were longer in Momiroman than in Teqing (Table 1).

NSC dynamics in culm of main stem

There was a clear difference in NSC accumulation pattern of the culm at pre-heading stage between years (Figure 2). In both varieties, the NSC accumulation of the culm was higher in 2019 than in 2018 from 21 to 8 DBH, but conversely was higher in 2018 than 2019 from 8 DBH to heading mainly due to the different solar radiation conditions. The varietal differences in NSC accumulation in the culm were observed in this period of relatively higher solar radiation in 2018 and 2019. In this period, NSC accumulation was greater in Teqing than in Momiroman; consequently the amount of NSC in culm at heading was higher in Teqing than in Momiroman in both years. The NSC accumulation of
Figure 1. Changes in each internode length of main stem. Bars represent standard error (n = 3).

Table 1. Morphological traits in each part of internode and leaf sheath of main stem.

| Year | Variety | Culm diameter (mm) | Internode length (mm) | Leaf sheath length (mm) |
|------|---------|--------------------|-----------------------|------------------------|
|      |         | 5th 4th 3rd 2nd 1st | 5th 4th 3rd 2nd 1st | 5th 4th 3rd 2nd 1st |
| 2018 | Momiroman | 5.79 5.31 5.16 4.90 3.02 | 20 59 122 216 421 | 231 261 287 308 407 |
|      | Teqing   | 6.63 6.07 5.30 4.81 3.04 | 49 89 114 169 345 | 200 209 226 255 363 |
| 2019 | Momiroman | 5.67 5.41 5.02 4.79 3.06 | 28 68 133 221 426 | 227 278 289 311 404 |
|      | Teqing   | 6.39 6.03 5.58 4.66 3.03 | 53 104 165 190 362 | 226 215 216 251 355 |
| mean | Momiroman | 5.73 5.36 5.09 4.85 3.04 | 24 63 127 219 424 | 229 270 288 309 405 |
|      | Teqing   | 6.51 6.05 5.44 4.74 3.04 | 51 97 140 180 353 | 213 212 221 253 359 |

ANOVA

| Year | Variety | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. |
|------|---------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|
|      | Momiroman | ***  | ***  | **   | **   | *    | **   | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. |
|      | Teqing   | n.s. | n.s. | *    | **   | n.s. | ***  | ***  | ***  | ***  | ***  | ***  | ***  | ***  | ***  | ***  |

Y × V

| | Momiroman | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. |
| | Teqing   | n.s. | n.s. | *    | **   | n.s. | ***  | ***  | ***  | ***  | ***  | ***  | ***  | ***  | ***  | ***  |

*, ** and *** significant at the 0.05, the 0.01 and the 0.001 level; n.s., not significant (n = 3).

Figure 2. Changes of non-structural carbohydrates (NSC) content in culm of main stem at pre- and post-heading stages. Bars represent standard error (n = 3).
the culm at post-heading stage continued until 6 DAH except for Teqing in 2018.

With respect to each segment of internodes, the NSC accumulation occurred in order from the lower internode (Figure 3) and the NSC concentration at heading stage was higher in the lower internode regardless of environment and genotype (Supplemental Figure S2). The 4th and 5th internodes in Teqing accumulated more NSC than in Momiroman (Figure 3), which led to the varietal difference in the amount of NSC in the entire culm at heading stage (Figure 2). The NSC accumulation of the 1st and 2nd internodes mainly started from 8 DBH and continued until 6 DAH, although the 4th and 5th internodes accumulated little NSC after heading in both varieties. The amounts of NSC accumulated in the 1st and 2nd internodes were greater in Momiroman than in Teqing, which is an opposite result to that for the 4th and 5th internodes (Figure 3). The NSC remobilization tended to occur earlier in the lower than the upper internode in both varieties as did the onset of NSC accumulation (Supplemental Figure S2).

**Dynamics of dry matter without NSC in culm of main stem**

The dry matter without NSC (residual components; RC) was calculated by subtracting NSC from dry matter weight. There were varietal differences in RC production of each internode during the period when solar radiation was higher in each year. In 2018, from 8 DBH to heading, Teqing showed an increase of RC in all internodes, but Momiroman showed no increase in the 4th and 5th internodes (Supplemental Figure S3), similar to the pattern of NSC changes (Figure 3). In 2019, the RC increase of the 4th and 5th internodes was greater in Teqing than in Momiroman during the period from 21 to 8 DBH. At post-heading stage, the RC production of the 1st and 2nd internodes was higher in Momiroman and the maximum amounts of RC in the both internodes were both about 120 mg more for Momiroman than Teqing, respectively. During the period from 21 DBH to 6 DAH, there were significant correlations between the NSC change rate and the RC change rate in each internode (Figure 4), which means that internode development coincided with NSC accumulation. The slope of each regression line showed

![Figure 3](https://example.com/figure3.png)

**Figure 3.** Changes of non-structural carbohydrates (NSC) content in each internode of main stem at pre- and post-heading stages. Bars represent standard error (n = 3).
the utilization ratio of RC and NSC. For example, the slope of the regression line for the 5th internode was about 0.98, which means that the half of the dry matter partitioned to the 5th internode was used for NSC accumulation during the period from 21 DBH to 6 DAH. The higher was the internode, the greater was the slope of the regression line. Particularly, the 1st internode showed the greatest amount of partitioned dry matter used for RC.

 Declines in RC were observed in all segments of internodes (Supplemental Figure S3), and the reduction ratio [the ratio of the decline amount (ΔRC), calculated by subtracting the minimum amount from the maximum amount, to the maximum amount of RC] was higher in lower than upper internodes for both varieties (Table 2). The reduction ratio and the decline amount in the 4th and 5th internodes were

Figure 4. Relationship between non-structural carbohydrates (NSC) accumulation rate and residual components (RC) accumulation rate in each internode of main stem. ΔNSC, calculated from the difference in NSC between two adjacent sampling times. ΔRC, calculated from the difference in NSC between two adjacent sampling times. The sampling times are 21 days before heading (DBH), 14 DBH, 8 DBH, heading and 6 days after heading in 2018 and 2019. If it is no changes in NSC and RC, it is excluded. ** and ***, significant at the 0.01 and the 0.001 level.

Table 2. Remobilization traits of residual components in each part of internode of main stem.

| Year | Variety | 5th | 4th | 3rd | 2nd | 1st | 5th | 4th | 3rd | 2nd | 1st |
|------|---------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 2018 | Momiroman | 35.8 | 35.1 | 82.3 | 55.7 | 32.2 | 0.30 | 0.19 | 0.29 | 0.17 | 0.09 |
|      | Teqing  | 100.5 | 94.3 | 76.6 | 32.0 | 32.4 | 0.44 | 0.38 | 0.33 | 0.16 | 0.13 |
| 2019 | Momiroman | 32.2 | 37.6 | 28.0 | 4.5 | 17.7 | 0.23 | 0.18 | 0.11 | 0.01 | 0.05 |
|      | Teqing  | 91.6 | 76.7 | 84.0 | 44.5 | 24.8 | 0.47 | 0.32 | 0.30 | 0.18 | 0.10 |
| mean | Momiroman | 34.0 | 36.3 | 55.1 | 30.1 | 24.9 | 0.26 | 0.18 | 0.20 | 0.09 | 0.07 |
|      | Teqing  | 96.0 | 85.5 | 80.3 | 38.2 | 28.6 | 0.46 | 0.35 | 0.31 | 0.17 | 0.11 |
| ANOVA | Year | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. |
|      | Variety | *** | * | n.s. | n.s. | n.s. | *** | * | n.s. | n.s. | n.s. |
|      | Y × V  | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. |

aΔRC, apparent remobilization amount of residual components (RC), calculated from the difference in RC between the maximum amount and the following minimum amount at pre- and post-heading stages. b Ratio of decline in RC, calculated by dividing ΔRC by the maximum amount of RC. *, **, *** significant at the 0.05 and the 0.001 level; n.s., not significant (n = 3).
significantly higher in Teqing than in Momiroman. Interestingly, in Teqing, the amount of RC decline in the 4th and 5th internodes was comparable to that of NSC (Supplemental Table S2).

**NSC dynamics in leaf sheath of main stem**

In the entire leaf sheaths of the main stem, unlike the culm, NSC decline occurred during the period of lower solar radiation at the pre-heading stage in both 2018 and 2019 (Figure 5). During the period from 8 DBH to heading in 2018 and from 14 to 8 DBH in 2019, i.e. higher solar radiation conditions, the NSC accumulation pattern differed between varieties. Teqing accumulated more NSC in this period than Momiroman, and so also accumulated more NSC than Momiroman at heading, although there was no significant varietal difference in 2019. The leaf sheath exhibited a rapid decline of NSC.

![Figure 5](image-url)

**Figure 5.** Changes of non-structural carbohydrates (NSC) content in leaf sheath of main stem at pre- and post-heading stages. Bars represent standard error (n = 3).

![Figure 6](image-url)

**Figure 6.** Changes of non-structural carbohydrates (NSC) content in each part of leaf sheath of main stem at pre- and post-heading stages. Bars represent standard error (n = 3).
after heading compared to the culm in both years (Figures 2, Figures 5).

The leaf sheath had already stored NSC at the young panicle formation stage, especially in the lower leaf sheath (Figure 6). The concentration of NSC was larger in the lower than the upper leaf sheath before heading as well as in the internode (Supplemental Figure S4). Teqing accumulated more NSC than Momiroman in the other segments except for the 1st leaf sheath during the period when solar radiation was relatively high in each year (Figure 6). The continuous NSC accumulation after heading, as seen in the upper internode, did not occur in each segment of the leaf sheath.

Dynamics of dry matter without NSC in leaf sheath of main stem

The maximum amounts of RC were greater in Momiroman than in Teqing in the 1st, 2nd and 3rd leaf sheaths (Supplemental Figure S5). The RC production showed similar trends for both varieties during the period from 8 DBH to heading in 2018 (Supplemental Figure S5), although the changes in NSC significantly differed between varieties (Figure 6). However, there were significant differences between varieties during the period from 15 to 8 DBH in 2019 (Supplemental Figure S5). In this period, Teqing showed increases in RC for other segments including the 1st leaf sheath, but Momiroman showed increases in only the 1st leaf sheath and with a greater increase than for Teqing. This RC production difference had a similar pattern to the NSC change.

In both varieties, all segments of the leaf sheaths showed RC decline at pre- and post-heading stages, although re-accumulation occurred in some segments, such as the 1st leaf sheath in 2019 (Supplemental Figure S5). The RC decline amount and reduction ratio appeared to differ among segments, but the difference was not as remarkable as the internodes (Tables 2, 3).

The reduction ratio tended to be higher in Teqing than Momiroman in all segments of the leaf sheaths (Table 3).

Discussion

Lower parts of stems function as major sink organs related to NSC accumulation

The lower internode was the main storage organ of NSC before heading (Figure 3; Supplemental Figure S2). In the culm of wheat, the increase in dry matter and water-soluble carbohydrates in internodes arises from the lower segments, which function as main storage organs at the pre-heading stage (Bahman Ehdaie et al., 2006b; Chen et al., 2014; B. Ehdaie et al., 2006a; H. Li et al., 2013). In general, internode elongation of grass species occurs sequentially from lower to upper nodal segments, making it possible for lower internodes to function earlier as sink organs and ensure sufficient time to accumulate NSC during the pre-heading stage.

More NSC was stored in the culms and leaf sheaths at full-heading or heading stages in Teqing than in Momiroman, which coincided with the results of Okamura et al. (2018), although there was no difference between varieties in leaf sheaths in 2019 (Figures 2, Figures 5). Previous reports indicated that NSC accumulation at pre-heading stage is related to crop growth rate for two weeks before heading (Takai et al., 2006) and the number of days to heading (Nagata et al., 2002); however, there were no significant differences in dry matter production ability at pre-heading stage and in the heading date among varieties used in this study (Supplemental Figure S6; Supplemental Table S1). Thus, it can be assumed that the varietal differences related to NSC accumulation are mainly due to differences in assimilate partitioning among organs or positions in an organ, and the utilization of the partitioned assimilates.

In the culm, this varietal difference in NSC accumulation clearly appeared during the relatively higher solar

Table 3. Remobilization traits of residual components in each part of leaf sheath of main stem.

| Year | Variety   | ΔRC * (mg) | Ratio of decline in RC b |
|------|-----------|------------|--------------------------|
|      |           | 5th | 4th | 3rd | 2nd | 1st | 5th | 4th | 3rd | 2nd | 1st | 5th | 4th | 3rd | 2nd | 1st |
| 2018 | Momiroman | 130.2 | 98.5 | 98.9 | 92.1 | 48.3 | 0.36 | 0.23 | 0.23 | 0.19 | 0.08 |      |      |      |      |      |      |
|      | Teqing    | 70.1  | 80.1 | 80.6 | 77.3 | 57.4 | 0.25 | 0.24 | 0.24 | 0.22 |      |      |      |      |      |      |
| 2019 | Momiroman | 39.4  | 76.2 | 74.1 | 70.8 | 64.2 | 0.14 | 0.21 | 0.18 | 0.16 | 0.11 |      |      |      |      |      |      |
|      | Teqing    | 99.8  | 114.3 | 115.3 | 85.8 | 90.7 | 0.37 | 0.32 | 0.31 | 0.24 | 0.22 |      |      |      |      |      |      |
| mean | Momiroman | 84.8  | 87.4 | 86.5 | 81.5 | 56.3 | 0.25 | 0.22 | 0.20 | 0.18 | 0.10 |      |      |      |      |      |      |
|      | Teqing    | 85.0  | 97.2 | 98.0 | 81.6 | 74.1 | 0.31 | 0.28 | 0.28 | 0.23 | 0.18 |      |      |      |      |      |      |
| ANOVA | Year | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. |      |      |      |      |      |      |
|      | Variety | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. |      |      |      |      |      |      |
|      | Y × V    | *     | n.s. | *     | n.s. | n.s. | **   | n.s. | n.s. | n.s. | n.s. |      |      |      |      |      |      |

*ΔRC, apparent remobilization amount of residual components (RC), calculated from the difference in RC between the maximum amount and the following minimum amount at pre- and post-heading stages. b Ratio of decline in RC, calculated by dividing ΔRC by the maximum amount of RC. * and **, significant at the 0.05 and the 0.01 level; n.s., not significant (n = 3).
radiation conditions in each year (Figure 2; Supplemental Figure S1), and most of this difference was attributed to varietal difference in the accumulation ability in the lower internodes (Figure 3). Teqing showed superior NSC accumulation ability in the lower internodes compared with Momiroman (Figure 3). The morphological size is considered to be related to the NSC accumulation ability in internodes (B. Ehdaie et al., 2006a). The lower internodes of Teqing showed greater length, diameter and storage capacity than those of Momiroman (Table 1). Thus, it is considered that larger lower internodes are one of factors for high NSC accumulation in culms at the pre-heading stage.

The lower segments of leaf sheaths accumulated more NSC than upper segments (Figure 6; Supplemental Figure S4), meaning that the lower segments were major storage organs in the pre-heading stage as well as the internodes. Wada et al. (2017) reported similar results using two rice cultivars (Momiroman and Hokuriku193) at one time point of heading stage. However, Watanabe et al. (1997) reported that upper leaf sheaths are specialized to accumulate more starch and soluble sugars than lower leaf sheaths. These inconsistent results can be explained by differences in tillering traits among varieties used in the various experiments. Watanabe et al. (1997) used Musashikogane, a japonica variety developed in Japan and defined as partial panicle number type (Hiraoka et al., 1992), which has more panicles than Teqing and Momiroman. Adriani et al. (2016) indicated that tiller emergence and growth of the main stem compete for C resources, using near-isogenic lines with quantitative trait loci related to the traits of early down-regulation of tillering. Therefore, NSC accumulated temporarily in the lower leaf sheath could be more actively utilized for tiller emergence until the end of vegetative period especially in the variety with greater panicle number. However, this varietal difference of NSC amount stored in the leaf sheath was not explained by the morphological trait of leaf sheath length (Table 1). The NSC accumulation in leaf sheath occurs locally at the side of the node (Ishimaru et al., 2004). In the present study, the localization of NSC accumulation occurred mainly in the leaf sheath, but little in the internode (Supplemental Figure S7). Therefore, not the length but some morphological trait at the side of node, such as thickness, might be important for leaf sheath NSC accumulation. Further research is required on the relationship between NSC accumulation and morphological traits of the leaf sheath. Also, morphological traits of lower parts of stem should be investigated in terms of the relationship with lodging resistance, one of important traits for high-yielding rice varieties (Islam et al., 2007; Zhang et al., 2014).

**Competitive relationship among sink organs at pre- and post-heading stages**

During the period from young panicle formation to heading, the panicle, culm and flag leaf sheath (i.e. 1st leaf sheath) simultaneously act as sinks, with the culm having the greatest sink ability (Fujita & Yoshida, 1984). The 1st and 2nd internodes and 1st leaf sheath were actively developed and formed during this period (Figure 1; Supplemental Figure S3; Supplemental Figure S5). The rapid elongation of the upper internodes and leaf sheaths at pre- and post-heading stages requires a large amount of C resources for structural components (SC). In the carbohydrate partitioning of stems, a negative correlation between NSC and SC has been reported for some grass species (Arai-Sanoh et al., 2011). Furthermore, a tall rice variety, with bigger culms and leaf sheaths, had more SC (i.e. starch weight subtracted from dry matter weight) and less starch in the leaf sheaths and culms than a short variety (Fujita & Yoshida, 1984). In the present study, compared with Teqing, more RC was needed by Momiroman to develop and form the upper internodes and 1st leaf sheath (Supplemental Figure S3; Supplemental Figure S5). This difference in investment of C resources for the upper parts of stems would lead to varietal differences in NSC accumulation in the culm and leaf sheath. Thus, it should be possible to increase NSC stored in stems by shortening the upper parts of stems and so mitigating its competition for C resources.

There were the varietal differences in the RC partitioning among segments of the internodes and leaf sheaths. Under the higher solar radiation conditions, Teqing partitioned more RC to lower internodes in both years and to the lower leaf sheath in only 2019 (Supplemental Figure S3; Supplemental Figure S5). The RC stored in the lower segments of the internodes and leaf sheaths showed the trait of easy decline and, in particular, the lower internodes in Teqing showed a greater rate of decline (Tables 2, 3). It has been shown that the NSC is not the only remobilized component of stems. (1–3, 1–4)-β-glucan of hemicellulose component among the cell wall components mainly breaks down in the lower internodes and leaf sheaths at the early ripening stage (Baba et al., 2001; Nemoto et al., 2004). Arai-Sanoh et al. (2013) indicated that indica-dominant varieties (Takanari and Shangquichao) have a trait of greater reduction ratio in (1–3, 1–4)-β-glucan and suggested that remobilization of (1–3, 1–4)-β-glucan in stems contributes to grain filling. Therefore, the higher RC reduction ratio of the lower
internodes in Teqing might be due to the ease of breakdown of (1–3, 1–4)-β-glucan. In addition, stems store not only C resources but also nitrogen (N), phosphorus (P) and magnesium (Mg), and remobilize these to panicles (Yoneyama et al., 1989). It was reported that the semi-dwarf indica variety (Suweon258) with superior NSC remobilization traits showed higher partitioning of N, P and Mg to the panicles from the stems (Yoneyama et al., 1989). In a study of the N partitioning among organs, indica variety (Takanari) stored more N in stems at about 10 DAH and remobilized more N to panicles than japonica variety (Nipponbare) (Ida et al., 2009). With respect to the P partitioning among organs, the stem is the main P storage organ at heading stage among vegetative organs (Liu & Zhu, 1996) and the stored P is remobilized to panicles during the 4 weeks after heading (Julia et al., 2016). Therefore, especially during the period from young panicle formation to heading, stems function as storage organs not only of C but also of various nutrients. The differences in the RC reduction ratio observed among segments of internodes and leaf sheaths in the present study indicate that further research concerning differences in remobilized components other than NSC is required.

Transition from sink to source in each segment of internodes and leaf sheath

The culm and the leaf sheath differed in remobilization traits (Figures 2, Figures 5). In a 14C tracer experiment, Fujita and Yoshida (1984) found that starch accumulation ability declined in the leaf sheath from about 2 weeks to 1 week before heading, while simultaneously increasing in the culm. Furthermore, Nagata et al. (2001) reported that the decline of NSC stored in the leaf sheath was accelerated by shading treatment for about 20 days before heading, with no decline observed in the culm, consistent with our results. Thus, compared with the internode, the leaf sheath more easily changed from sink to source organ, especially in photosynthesis-limiting conditions.

The starting time of NSC remobilization of stems at post-heading stage is an important factor related to grain filling (Wada et al., 2017; Yoshinaga et al., 2013). Notably, in the present study, not the leaf sheaths but the culms delayed NSC remobilization among stems and there was clear varietal difference for culms (Figures 2, Figures 5). It is possible to explain this varietal difference from the composition balance of each segment of internodes. The NSC remobilization pattern, as well as NSC accumulation, differed among segments of internodes (Figure 3, Supplemental Figure S2). In both varieties, NSC remobilization at post-heading stage was later in the upper than in the lower internodes (Supplemental Figure S2). Okawa et al. (2002) reported that the culm continued to function as a sink organ for a while after heading, and the timing of C distribution switching from culm to panicle was about 10 DAH. Furthermore, Okawa et al. (2003) indicated that the continuous NSC accumulation in the upper internodes after heading would result from temporary accumulation of surplus assimilate that was not consumed for the development of grains and internodes. In the present study, Momiroman accumulated more NSC in the upper internodes for 6 days after heading than Teqing (Figure 3), although Momiroman had greater consumption with the active elongation and formation of the upper internodes during this period (Figure 1; Table 1; Supplemental Figure S3). Our results indicated that NSC accumulation and internode formation occurred synchronously (Figure 4), suggesting that internode elongation and formation required more carbohydrates than the demand and its surplus consequently was accumulated as storage carbohydrates. Therefore, it seems likely that the variety with relatively larger upper and smaller lower internodes (Momiroman) tend to have traits to delay the onset of NSC remobilization, while the variety with adverse composition balance (Teqing) showed earlier NSC remobilization traits.

Some studies explained the delay in NSC remobilization in Momiroman physiologically, using the 3rd internode as a representative. Wada et al. (2017) suggested that the starch stored in internodes of Momiroman is difficult to degrade mainly due to the low expression of the gene for glucan water dikinase1, which is related to starch degradation. Meanwhile, Okamura et al. (2018) suggested that there are problems in the step of, not starch degradation, but sucrose transport in the NSC remobilization processes. In the present study, it seems that starch degradation in the 3rd, 4th and 5th internodes after heading was more facilitated in Teqing than in Momiroman, although there were no varietal difference in the 1st and 2nd internodes (Supplemental Figure S8). While, the soluble sugar content after heading was higher in Momiroman than in Teqing, especially in the 2nd internode (Supplemental Figure S8), which implies a lower sucrose remobilization efficiency in Momiroman. Although more exhaustive analysis, including measurements of flowering pattern (Yabe et al., 2018) and/or grain growth (Okamura et al., 2018), will be needed to elucidate the metabolic and physiological mechanisms of NSC remobilization in rice stems, the present results suggest that limiting factors in the NSC remobilization process are different for each segment of internodes, and thus it is important to analyse stems by segments.
Conclusion

We determined that each segment of internodes and leaf sheaths had different traits in NSC or RC dynamics at pre- and post-heading stages in common among two varieties. The lower parts of the stems functioned as the major sink organs of NSC at pre-heading stage and as major source organs of NSC and RC at post-heading. The morphologically larger lower internodes could store more NSC and RC. In contrast, the upper parts of stems needed RC, which is inferior in regard to remobilization traits, for their development and formation during the period when NSC was actively accumulating in stems. Compared to shorter examples, formation of the longer period when NSC was actively accumulating in stems. The NSC stored in the upper internodes showed traits of delayed remobilization compared with lower internodes, which led to delays in NSC remobilization in the entire culm. Consequently, we suggest from the comparison among the potential as sink organs of remobilized components and as source organs for grain development.

Acknowledgments

We express our gratitude to the staff at ISAS, University of Tokyo, for technical support of this study in the cultivation and management of rice.

Disclosure statement

No potential conflict of interest was reported by the author(s).

References

Adriani, D. E., Lafarge, T., Dardou, A., Fabro, A., Clément-Vidal, A., Yahya, S., Dingkuhn, M., & Luquet, D. (2016). The qTSN positive effect on panicle and flag leaf size of rice is associated with an early down-regulation of tillering. Frontiers in Plant Science, 6(JAN2016), 1–17. https://doi.org/10.3389/fpls.2015.01197

Arai-Sanoh, Y., Idia, M., Zhao, R., Nishitani, K., Yoshinaga, S., Takai, T., Nakano, H., Iwasawa, N., & Kondo, M. (2013). Varietal differences in cell wall β-[1→3]-β-[3→4]-glucan and nonstructural carbohydrate in rice stems during the grain filling stage. Plant Production Science, 16(4), 335–341. https://doi.org/10.1626/pps.16335

Arai-Sanoh, Y., Idia, M., Zhao, R., Yoshinaga, S., Takai, T., Ishimaru, T., Maeda, H., Nishitani, K., Terashima, Y., Gau, M., Kato, N., Matsuoka, M., & Kondo, M. (2011). Genotypic variations in non-structural carbohydrate and cell-wall components of the stem in rice, sorghum, and sugar cane. Bioscience, Biotechnology, and Biochemistry, 75(6), 1104–1112. https://doi.org/10.1271/bbb.110009

Baba, Y., Ishida, Y., Oda, M., Iiyama, K., & Akita, S. (2001). Decomposition of (1→3)-β-glucan and expression of the (1→3)-β-glucanase gene in rice stems during ripening. Plant Production Science, 43(1), 230–234. https://doi.org/10.1626/pps.4.230

Chen, W., Deng, X. P., Eneji, A. E., Wang, L. L., Xu, Y., & Cheng, Y. J. (2014). Dry-matter partitioning across parts of the wheat internode during the grain filling period as influenced by fertilizer and tillage treatments. Communications in Soil Science and Plant Analysis, 45(13), 1799–1812. https://doi.org/10.1080/00103624.2014.907918

Cock, J. H., & Yoshida, S. (1972). Accumulation of 14C-labeled carbohydrate before flowering and its subsequent redistribution and respiration in the rice plant. Proceedings Crop Science Society of Japan, 41, 226–234. http://www.mendeley.com/research/geology-volcanic-history-eruptive-style-yakedake-volcano-group-central-japan/

Ehdai, B., Alloush, G. A., Madore, M. A., & Waines, J. G. (2006a). Genotypic variation for stem reserves and mobilization in wheat: I. Postanthesis changes in internode dry matter. Crop Science, 46(2), 735–746. https://doi.org/10.2135/cropsci2005.04-0033

Ehdai, B., Alloush, G. A., Madore, M. A., & Waines, J. G. (2006b). Genotypic variation for stem reserves and mobilization in wheat: II. Postanthesis changes in internode water-soluble carbohydrates. Crop Science, 46(5), 2093–2103. https://doi.org/10.2135/cropsci2006.01013

FAO. (2017). Food and Agriculture Organization of the United Nations, FAOSTAT, data, food balance, new food balances. accessed 6 July 2020. http://www.fao.org/faostat/en/#data/FBS

Fu, J., Huang, Z., Wang, Z., Yang, J., & Zhang, J. (2011). Pre-anthesis non-structural carbohydrate reserve in the stem enhances the sink strength of inferior spikes during grain filling of rice. Field Crops Research, 123(2), 170–182. https://doi.org/10.1016/j.fcr.2011.05.015

Fujita, K., & Yoshida, S. (1984). Partitioning of photosynthates between growing panicle and vegetative parts before anthesis. Soil Science and Plant Nutrition, 30(4), 509–518. https://doi.org/10.1080/00380768.1984.10434718

Hiraoaka, K., Yoshida, S., Takebe, M., & Yoneyama, T. (1992). Physiological characteristics of high-yielding rice varieties(III) effects of air temperature and sunshine radiation on the dry matter production and uptake and partitioning of N and P in suweon 258. Japanese Society of Soil Science and Plant Nutrition, 63(2), 177–183. https://doi.org/10.20710/doi.63.2_177

Ida, M., Ohsugi, R., Sasaki, H., Aoki, N., & Yamagishi, T. (2009). Contribution of nitrogen absorbed during ripening period to grain filling in a high-yielding rice variety, takanari. Plant Production Science, 12(2), 176–184. https://doi.org/10.1626/pps.12.176

IRRI (2016). Leading innovations annual report 2016. ISSN 0074–7793. International Rice Research Institute, 1–40.

Ishikawa, T., Akita, S., & Li, Q. (1993). Relationship between content of nonstructural carbohydrates before panicle initiation stage and grain yield in rice (Oryza sativa L.). Japanese Journal of Crop Science, 62(1), 130–131. https://doi.org/10.1626/jcs.62.130

Ishimaru, K. (2003). Identification of a locus increasing rice yield and physiological analysis of its function. Plant Physiology, 133(3), 1083–1090. https://doi.org/10.1104/pp.103.027607
Ishimaru, K., Kashiwagi, T., Hirotsu, N., & Madoka, Y. (2005). Identification and physiological analyses of a locus for rice yield potential across the genetic background. *Journal of Experimental Botany*, 56(420), 2745–2753. https://doi.org/10.1093/jxb/eri267

Ishimaru, K., Kosone, M., Sasaki, H., & Kashiwagi, T. (2004). Leaf contents differ depending on the position in a rice leaf sheath during sink-source transition. *Plant Physiology and Biochemistry*, 42(11), 855–860. https://doi.org/10.1016/j.plaphy.2004.10.008

Islam, M. S., Peng, S. B., Vispers, R. M., Ereful, N., Bhuiya, M. S. U., & Julifquar, A. W. (2007). Lodging-related morphological traits of hybrid rice in a tropical irrigated ecosystem. *Field Crops Research*, 101(2), 240–248. https://doi.org/10.1016/j.fcr.2006.12.002

Julia, C., Wissuwa, M., Kretzschmar, T., Jeong, K., & Rose, T. (2016). Phosphorus uptake, partitioning and redistribution during grain filling in rice. *Annals of Botany*, 118(6), 1151–1162. https://doi.org/10.1093/aob/mcw164

Khush, G. S. (2005). What it will take to Feed 5.0 Billion Rice consumers in 2030. doi.org/10.1007/s11103-005-2159-5

Okamura, M., Arai-Sanoh, Y., Yoshida, H., Mukouyama, T., Adachi, S., Yabe, S., Nakagawa, H., Tsutsumi, K., Taniguchi, Y., Kobayashi, N., & Kondo, M. (2018). Characterization of high-yielding rice cultivars with different grain-filling properties to clarify limiting factors for improving grain yield. *Field Crops Research*, 219(2), 139–147. https://doi.org/10.1016/j.fcr.2018.01.035

Okamura, M., Hirose, T., Hashida, Y., Yamagishi, T., Ohsugi, R., & Aoki, N. (2013). Starch reduction in rice stems due to a lack of OsAGPL1 or OsAPL3 decreases grain yield under low irradiance during ripening and modifies plant architecture. *Functional Plant Biology*, 40(11), 1137–1146. https://doi.org/10.1071/FP13105

Okawa, S., Makino, A., & Mae, T. (2002). Shift of the major sink from the culm to the panicle at the early stage of grain filling in rice (oryza sativa L. cv. sasanishiki). *Soil Science and Plant Nutrition*, 48(2), 237–242. https://doi.org/10.1080/00380768.2002.10409196

Okawa, S., Makino, A., & Mae, T. (2003). Effect of irradiance on the partitioning of assimilated carbon during the early phase of grain filling in rice. *Annals of Botany*, 92 (3), 357–364. https://doi.org/10.1093/aob/mcg147

Oookawa, T., Naboruka, Y., Yamazaki, T., Suga, J., & Hirasawa, T. (2003). A comparison of the accumulation and partitioning of nitrogen in plants between two rice cultivars, akenohoshi and nipponbare, at the ripening stage. *Plant Production Science*, 6(3), 172–178. https://doi.org/10.1626/pps.6.172

Pan, J., Cui, K., Wei, D., Huang, J., Xiang, J., & Nie, L. (2011). Relationships of non-structural carbohydrates accumulation and translocation with yield formation in rice recombinant inbred lines under two nitrogen levels. *Physiologia Plantarum*, 141(4), 321–331. https://doi.org/10.1111/j.1399-3054.2010.01441.x

Powell, N., Ji, X., Ravash, R., Edlington, J., & Dolferus, R. (2012). Yield stability for cereals in a changing climate. *Functional Plant Biology*, 39(7), 539–552. https://doi.org/10.1071/FP12078

Samonte, S. O. P. B., Wilson, L. T., McClung, A. M., & Tarpley, L. (2001). Seasonal dynamics of nonstructural carbohydrate partitioning in 15 diverse rice genotypes. *Crop Science*, 41 (3), 902–909. https://doi.org/10.2135/cropsci2001.413902x

Takai, T., Matsuura, S., Nishio, T., Ohsumi, H., Shiraiwa, T., & Horie, T. (2006). Rice yield potential is closely related to crop growth rate during late reproductive period. *Field Crops Research*, 96(2–3), 328–335. https://doi.org/10.1016/j.fcr.2005.08.001

Wada, H., Masumoto-Kubo, C., Tsutsumi, K., Nonami, H., Tanaka, F., Okada, H., Erra-Balsells, R., Hiraoka, K., Nakashima, T., Nakamura, Y., & Morita, S. (2017). Turgor-responsive starch phosphorylation in Oryza sativa stems: A primary event of starch degradation associated with grain-filling ability. *PLoS ONE*, 12(7), 7. https://doi.org/10.1371/journal.pone.0181272

Watanabe, Y., Nakamura, Y., & Ishii, R. (1997). Relationship between starch accumulation and activities of the related enzymes in the leaf sheath as a temporary sink organ in rice (Oryza sativa). *Australian Journal of Plant Physiology*, 24(5), 563–569. https://doi.org/10.1071/PP96107

Wu, G., Wilson, L. T., & McClung, A. M. (1998). Contribution of rice tillers to dry matter accumulation and yield. *Agronomy Journal*, 90(3), 317–323. https://doi.org/10.2134/agronj1998.00021962009000030001x

Yabe, S., Nakagawa, H., Adachi, S., Mukouyama, T., Arai-Sanoh, Y., Okamura, M., Kondo, M., & Yoshida, H. (2018). Model analysis of genotypic difference in the variation of the

---

*PLANT PRODUCTION SCIENCE* 41
duration from heading to flower opening based on the flower position on a panicle in high-yielding rice cultivars. *Field Crops Research*, 223, 155–163. https://doi.org/10.1016/j.fcr.2018.04.013

Yamamoto, T., Yonemaru, J., Ebana, K., & Yano, M. (2010). Genome competition of Japanese high yield cultivars estimated by SNP typing array. *Breeding Research*, 12 (Ex. 1), 18.

Yang, J., Zhang, J., Wang, Z., Zhu, Q., & Wang, W. (2001). Remobilization of carbon reserves in response to water deficit during grain filling of rice. *Field Crops Research*, 71 (1), 47–55. https://doi.org/10.1016/S0378-4290(01)00147-2

Yoneyama, T., Fukuda, M., & Kouchi, H. (1989). Partitioning of carbon, nitrogen, phosphorus, potassium, calcium, and magnesium in a semidwarf high-yielding rice variety: Comparison with a conventional japonica variety. *Soil Science and Plant Nutrition*, 35(1), 43–54. https://doi.org/10.1080/00380768.1989.10434735

Yoshinaga, S., Takai, T., Arai-Sanoh, Y., Ishimaru, T., & Kondo, M. (2013). Varietal differences in sink production and grain-filling ability in recently developed high-yielding rice (Oryza sativa L.) varieties in Japan. *Field Crops Research*, 150, 74–82. https://doi.org/10.1016/j.fcr.2013.06.004

Zhang, J., Li, G. H., Song, Y. P., Liu, Z. H., Yang, C. D., Tang, S., Zheng, C. Y., Wang, S. H., & Ding, Y. F. (2014). Lodging resistance characteristics of high-yielding rice populations. *Field Crops Research*, 161, 64–74. https://doi.org/10.1016/j.fcr.2014.01.012