論文の内容の要旨

生産·環境生物学専攻
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何 海燕
指導教員　山岸徹助教授

論文題目：　**Spatial Variation in the Carbohydrate Accumulation in Rice Leaf Sheath and its Varietal Difference**
(イネ葉鞘における炭水化物蓄積の空間的変異とその品種間差)

Leaf sheath of rice accumulates non-structural carbohydrates (NSC) before heading and translocates them to the grain after heading, contributing 20% to the yield. NSC was effective in increasing the percentage of ripened grains, particularly when the plants suffered environmental stress such as high temperature during the reproductive stage. The low percentage of ripened grains significantly limited the yield of the new plant type lines (NPT). To improve NSC in the leaf sheaths, it is necessary to elucidate the mechanism of carbohydrate storage in a single leaf sheath. There was variation of starch content with the position in the leaf sheath in japonica varieties. Therefore, the spatial variation in the carbohydrate accumulation in the leaf sheath of various varieties was comprehensively investigated from the physiological, morphological, and molecular biological aspects. The third leaf sheaths from the top were separated into five segments with the same length and used for analysis.

1. Carbohydrate amounts in the different segments and the relationship with the enzyme activities

Spatial variation of starch accumulation along the leaf sheath was investigated.

Starch content increased from the apical to the basal segment in all 4 varieties. Before heading, the proportion of starch content in the basal segment to that in the whole leaf sheath varied from 35% in IR65598-112-2 (*tropical japonica*, NPT) to 50% in Nipponbare (*japonica*) and 60% in the other two (*indica*). In contrast, the proportion in the apical segment was 8% in IR65598-112-2 but 1% in the others. This high/low gradient in starch content was not determined by the amount of photoassimilate supply from the leaf blade. To confirm whether the low gradient is NPT- or *tropical japonica*-specific, more varieties were subjected to carbohydrate measurement. Only another NPT line and a parent of the NPT lines showed the segmental difference similar to that in IR65598-112-2. The low gradient was then considered to be an inheritable trait.

Now that the high/low gradient was not determined by the amount of photoassimilate supply, next the activities of the enzymes involved in starch synthesis were measured. During rapid starch accumulation, the activities of SuSy, AGPase, SS and BE increased toward the basal segment. This is consistent with the basipetally increasing starch content. The activities of BE and GBSS in the apical segment of IR65598-112-2 were higher than those of Nipponbare, and the activities of GBSS and AGPase in the basal segment the opposite. These corresponded well with the varietal difference of starch content between IR65598-112-2 and Nipponbare. In addition, the SS activity in the basal segment of Nipponbare was over 4-fold higher than that of IR65598-112-2 at the early stage though not thereafter. Thus, enzyme activities, particularly those of SS and GBSS, may be responsible for the spatial variation in starch amount in the leaf sheath and the varietal difference.

2. Starch granules distributed in the transverse sections of the segments

The spatial variation of starch accumulation across the transverse section was observed. The transverse section consists of the abaxial fraction, the septum and the adaxial fraction. Starch accumulation in the lower segments progressed as follows: starch granules firstly appeared in the parenchyma cells adjacent to the adaxial side of the abaxial vascular bundle; secondly spread both abaxially to the abaxial fraction and adaxially along the septum, and both the granule number in a cell and the granule size increased; meanwhile, starch granules appeared around the adaxial sclerenchyma; later extended abaxially along the septum and laterally along the adaxial fraction. In the apical segment, starch granules were stored only in the septum. In the transverse section, both the area of the starch-filled parenchyma cells and the granule density in the cells increased basipetally and showed the varietal difference. At the peak of starch accumulation, approximately half of the parenchyma cells in both the abaxial fraction and the septum, and few in the adaxial fraction were filled with starch granules in the basal segment of IR65598-112-2 whereas all parenchyma cells were so in Nipponbare.

The progress of starch accumulation in the lower segments showed a close relationship with both the abaxial vascular bundle and the adaxial sclerenchyma region. The abaxial vascular bundles were reported previously to transport the majority of the photoassimilate to the leaf sheath. It seems that the adaxial sclerenchyma region, where the monosaccharide transporter was found, may also supply substrate for starch synthesis. The abaxial fraction of the apical segment showed the morphology of the cells different to that of the parenchyma cells. It was also green and free of starch granules. These may be linked to the photosynthetic function. However, photosynthesis in the apical segment did not affect the segmental difference of starch concentration once the leaf sheath finished its elongation. The parenchyma cells in the lower segments degraded with the development of the leaf sheath, but the varietal difference of starch accumulation in the basal segment seems to bear no relation to it. The P/V ratio, which is defined as the area of the parenchyma cells to that of the abaxial vascular bundle, was higher in the basal segment of IR65598-112-2 than that of Nipponbare. The ratio may probably contribute to the varietal difference of total carbohydrate concentration in the basal segment.

3. Expression patterns of the genes regulating starch synthesis

The segmental, fractional and varietal differences of starch-synthesis gene expressions were compared with those of starch concentrations. The leaf sheath was separated into the abaxial fraction, the septum and the adaxial fraction. The fractional difference in carbohydrate concentration was first compared between IR65598-112-2 and Nipponbare. Starch concentration increased from the adaxial to the abaxial fraction in the basal segment of IR65598-112-2. The fractional difference is not so apparent in Nipponbare. Sucrose concentration, however, showed higher values in the adaxial fractions in both varieties. On the other hand, the fractional pattern of the AGPase activity was similar to that of starch. It seems that the activities of enzymes involved in starch synthesis, like that of AGPase, may regulate starch accumulation even at the fraction level, though other enzyme activities could not be measured due to the limited amount of the samples.

The expression levels of the mRNAs were quantified using RT-PCR technology. Among the genes encoding the starch synthesis-related enzymes, *AGPase-L1, -S1, GBSSII, SuSy 1, 2* and the eight *SS* showed detectable expression in the leaf sheath, while *AGPase-L2, -S2, GBSS I, SuSy 3* not. *SSII-2, III-1* and *III-2* were selected representing the different types of *SS* expression in the leaf sheath. The expression levels of all genes were higher in the basal than in the apical segment, particularly at early accumulation stage. At the beginning of starch accumulation, the expression levels of *SuSy1, SuSy2, GBSSII, AGPase-S1, -L1, SSIII-1, SSIII-2* increased abaxially in the basal segment; the expression levels of most genes in the basal segment were higher in Nipponbare than in IR65598-112-2, and those in the apical segment were the opposite. These gene expression patterns at the early stage corresponded well with the segmental, fractional and varietal differences of starch concentration.

In conclusion, the spatial variation of starch accumulation in the leaf sheath, which includes the segmental and the fractional variations, and its varietal difference were investigated. The activities of the enzymes involved in starch synthesis and the expression level of the corresponding genes showed the similar segmental, fractional and varietal differences particularly at the early stage. The segmental and varietal differences of starch accumulation were found to be also based on the morphological differences. The results obtained in this study will surely help to improve the varieties to accumulate more carbohydrate before heading and to obtain a higher yield.