Spatial variation of carbohydrate metabolism in rice leaf sheath

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Abstract

Four rice cultivars, Nipponbare, IR72, Takanari, and IR65598-112-2 (abr. IR655), were used to study carbohydrate metabolism in the third leaf sheath counted from top. Results show that sucrose and starch contents increase from the tip to the base segment in the third leaf sheath. However, the gradient of starch content differs greatly among cultivars. It shows a steeper pattern in the order: IR655<Nipponbare< Takanari<IR72. Comparing with other cultivars, the whole third leaf sheath of IR655 owns significantly lower starch concentration and two-fold higher sucrose concentration which changes similarly to starch, suggesting that sucrose also acts as a storage substrate in IR655. Among the four measured enzymes, the activity of starch synthase correlates very well with the starch concentration. A possible accumulation pattern across the transverse sections is also proposed through morphological observation using light microscope.

Media summary

Spatial variation of carbohydrate accumulation, as well as its difference among cultivars, is discovered within rice leaf sheath.

Key words

Adaxial, sclerenchyma, abaxial, vascular bundle, epidermis, bridge

Introduction

There are only a few studies on carbohydrate metabolism in rice leaf sheath, although starch stored in leaf sheath before anthesis plays an important role on rice yield formation. Carbohydrate remobilized from the leaf sheath and culm to grain could contribute to rice yield as much as 38% (Yoshida and Aha 1968). The leaf sheath, especially the third one, plays a more important role than the culm in the temporary storage of starch (Watanabe et al. 1997, Hirose et al. 1999). Only activity of starch synthase bound to the starch granule (GBSS) was reported to parallel the level of starch (Perez et al. 1971). Steady-state mRNA levels of ADPglucose pyrophosphorylase (AGPase), soluble starch synthase (STS), and branching enzyme coincide with a rapid starch accumulation (Hirose et al. 1999). Assay works of all the above literatures were done using the whole leaf sheath. However, the base of a leaf sheath accumulates much starch as reported by Togari and Sato (1954). To further elucidate the mechanism of carbohydrate metabolism in leaf sheaths, it is necessary to explore the possible mechanism within one leaf sheath.

Methods

In 2002, four cultivars, IR72, Takanari, IR655, and Nipponbare were cultivated (22 hills/m^2, one plant per hill). After the third leaf sheath (counting the flag leaf as the first leaf) finished its elongation, it was sampled from the main stem and divided into five segments with same length. Samples for carbohydrate assay were lyophilized and stored at room temperature. Samples for enzyme assay were frozen in liquid nitrogen and stored at -80?C. Carbohydrates were measured according to Bergmeyer and Bernt (1974), and enzyme activities to Nakamura et al (1989). In 2003, change of starch granules across the transverse sections in the third leaf sheath of Nipponbare and IR655 were observed on the day starch concentration peaked.

Results

Both starch and sucrose concentrations of the whole leaf sheath show little difference among Nipponbare, Takanari, and IR72. However, starch concentration in IR655 is only half of that in the other three cultivars (Fig. 1a), and sucrose concentration is two folds of the others (Fig. 1b). Furthermore, only in IR655, the temporal pattern of sucrose concentration is similar to that of starch concentration. This implies that sucrose in IR655 may also play a role in carbohydrate storage.

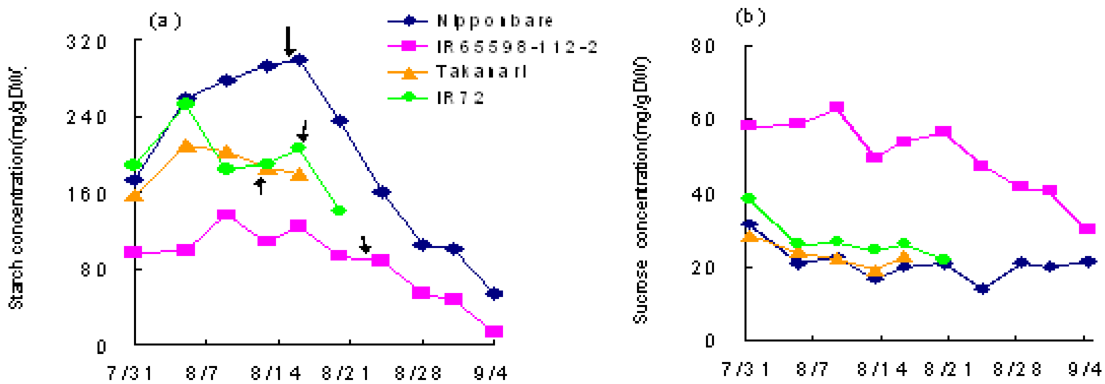

Fig. 1: Starch (a) and sucrose (b) concentrations in 4 cultivars changing with time. (heading date).

As for the spatial variation of starch and sucrose contents in the third leaf sheath, all cultivars show that both starch and sucrose contents increase from the tip to the base segment (Fig. 2). The gradient of starch level from tip to base, however, shows a sharper pattern in the order: IR655<Nipponbare<Takanari< IR72. Gradient of sucrose has a similar but duller pattern to that of starch.

The enzyme activities of SuSy (sucrose synthase), AGPase, STS and GBSS of Nipponbare and IR655 on one week before the peak of starch concentration are shown in Fig. 3. AGPase activities are higher in Nipponbare than in IR655. AGPase in both cultivars, SuSy in Nipponbare, and GBSS in IR655 show no consistent profile with starch. Interestingly, STS activities are always higher in the base segments; they correlate quite well with the starch concentrations. The above results suggest that STS is a pivotal factor influencing starch synthesis in rice leaf sheath.

Morphological observations show that the midrib part of the transverse section accumulates starch prior to the near edge part. In the tip segment, only few starch granules locates in the cells on the xylem side of the abaxial vascular bundle in Nipponbare, however, additional starch granules appear along the bridge between the inner and outer epidermis in IR655. In the middle segment, starch granules appear around the abaxial vascular bundle and the adaxial sclerenchyma, along the bridge and the outer epidermis in IR655, however, no granules appear around the schlerenchyma and little in the outer epidermis in Nipponbare.

In the base segment, granules appear in all the cells except that between the adaxial sclerenchyma, granules both in the middle of the bridge and between the vascular bundles in the outer epidermis are sparse. Based on the above observation, a possible progress of starch accumulation across the transverse section is proposed in Fig. 4.

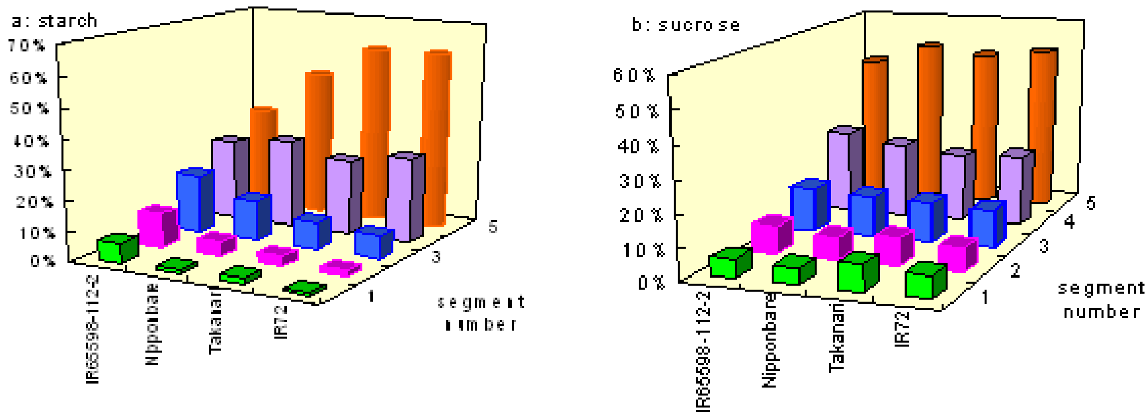

Fig. 2: Spatial variations in starch (a) and sucrose (b) contents in the third leaf sheath. (Percentage indicates average ratio of starch content in each segment to that in the total leaf sheath from day 7/31 to 8/16 since there is only little change of the ratio with time; 1, 3 and 5 point to the tip, middle and base segment respectively, the same is followed in Fig. 3.)

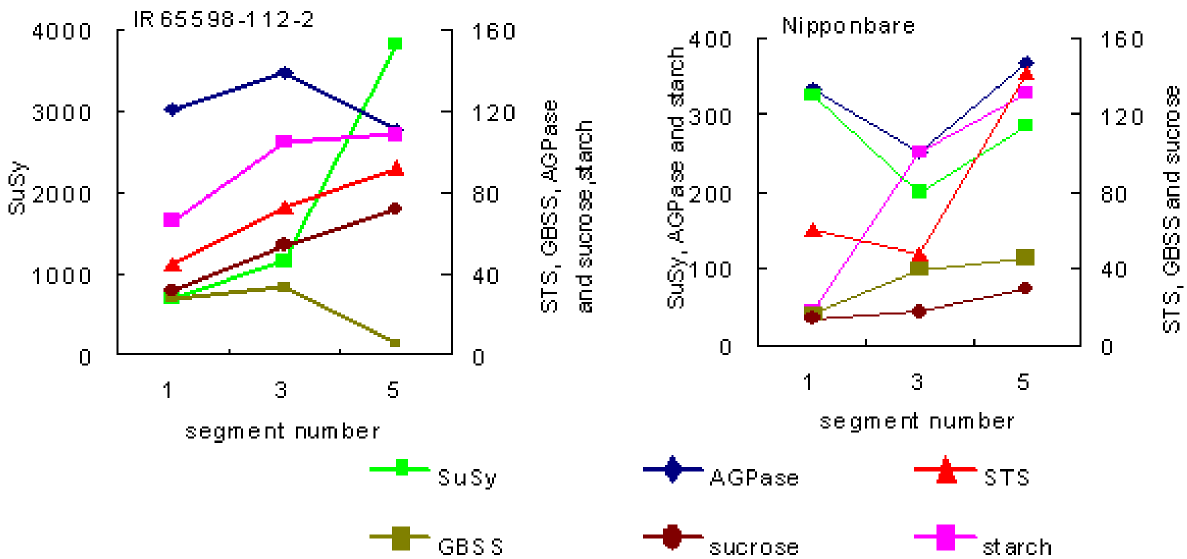

Fig. 3: Enzyme activities (nmol/min.gDW) of SuSy, AGPase, STS and GBSS in three segments of two cultivars.

Conclusion

Based on the above results, we conclude that the base segment of the leaf sheath plays the most important role in carbohydrate storage. Cultivars` difference, however, exists in the spatial variation pattern of carbohydrate accumulation within the leaf sheath. STS is proposed to be the pivotal enzyme affecting the cultivars` difference and spatial variation in starch accumulation. In IR655, sucrose may also act as a storage substrate. Starch granules accumulate across the tranverse section as illustrated in Fig. 4.

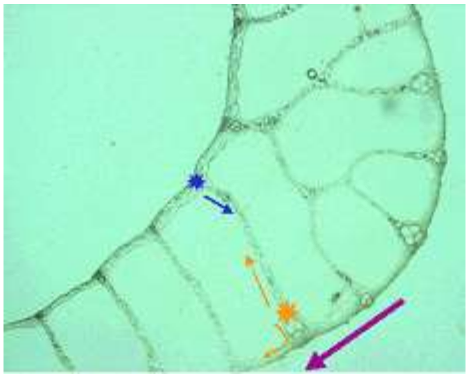

Fig. 4: Schematic illustration of starch accumulation progress across the transverse section. Starch granules accumulate from the midrib to the edge (purple arrow). Starch granules firstly appear in the cells on the xylem side of the abaxial vascular bundle(orange asterisk); secondly stretch both outward to the outer epidermis and inward along the bridge(orange arrows); Thirdly starch granules appear around the adaxial sclerenchyma(blue asterisk); fourthly extend outward along the bridge(blue arrow).

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