Starch Granules in Tissues of Rice Plants and Their Changes in Relation to Plant Growth

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The rice plant is a conspicuous cereal which accumulates many starch granules in its tissues.[3] The author carried out a series of studies on starch granules in rice plants in a period from 1954 to 1981.[1-10] Some of the major results will be presented in this paper.

Accumulation and distribution of starch granules in the tissues were microscopically observed after staining starch granules with I-KI solution or by using the Pass reaction.** For leaf sheaths and stem-internodes, where a large amount of starch granules accumulate in parenchyma, histochemical methods shown in Fig. 1 were employed to estimate the relative amount of starch in these organs.[10]

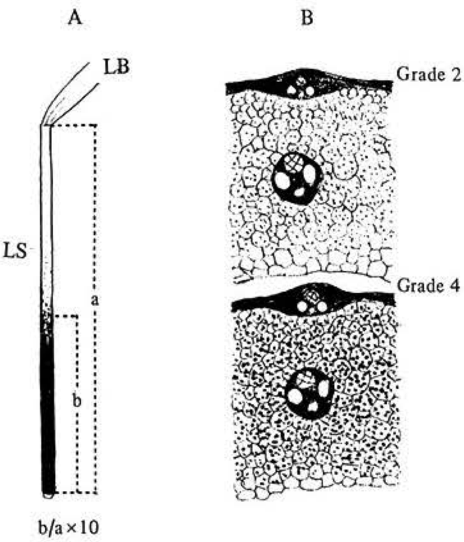

Fig. 1. Methods to estimate relative contents of starch in leaf sheath (A) and stem internode (B)

Note: Starch content in stem internode was graded from 0 to 5 based on the color density of starch-IKI complex, observed on hand-made cross sections of similar thickness. The sections were taken from upper, middle, and basal portions of each internode. Starch content in leaf sheath was calculated by a ratio, b/a × 10, where a = total length of leaf sheath, and b = length of the portion showing starch-IKI coloration.

Changes of starch contents in relation to plant growth

The organ which deposited the largest amount of starch was kernel, followed by internode, leaf-sheath, leaf-blade, and root in that order. In the latter two, starch deposit was normally very scarce. However in some cases when the "sink" for starch was severely damaged, they also deposited much starch.[3]

Starch amount was variable among tissues, and it changed with age and environment even in the same tissue.[1,2] A general trend of relative content of starch in the whole vegetative part of a plant through its life is expressed in Fig. 2. Young shoots contain a small amount of starch at an early heterotrophic stage at nursery bed, but abundant starch at the end of the nursery period, especially when soil nitrogen became less. Soon after transplanting, starch amount increased a little due to a limited nitrogen absorption

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** The details of plastid were observed by electron microscope

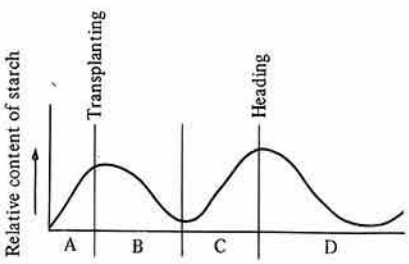

Fig. 2. Diagram showing relative contents of starch in vegetative portion of rice plants at different growth stages
A: Nursery stage
B: Tillering stage
C: Stem elongation—Heading stage
D: Ripening stage

and growth stagnation, but it soon began to decrease sharply because of rapid consumption of starch for tiller and root formation.[10] Young tillers with less than three leaves are nutritionally dependent on their respective mother culms, but after they formed more than three leaves they grow independently of mother culms and gradually deposit starch in the maturing parenchyma of leaf sheaths and internodes.[4] Main-stem and vigorous primary tillers formed from the lower part of the main-stem stored much starch at earlier growing stage, but its amount decreased as secondary and tertiary tillers rapidly emerged, because these late emerged tillers depend on early emerged tillers for nutrition until they form three expanded leaves. Then they become independent, and gradually store transitory starch in them. At the young panicle formation stage, all the tillers that emerged early or late, contained almost a similar amount of starch. Then their internodes elongated almost at the same rate, associated with a similar change of starch content (Fig. 3).[4]

After young panicles were formed, tillering ceased and root growth was limited, but photosynthesis continued. Therefore much transitory starch was gradually accumulated in the expanded, maturing parenchyma of leaf-sheath and stem-internode, reaching its peak before

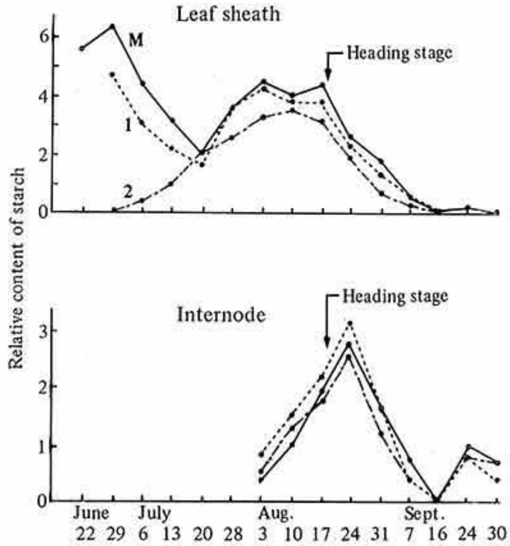

Fig. 3. Changes in starch content in main stem and tillers as related to plant growth
M: Main-stem
1: Primary tiller
2: Secondary tiller

or after heading (Fig. 2, 3). A decrease of starch amount in leaf-sheath at heading is caused by death of lower and older leaves.[4,10]

During the ripening period, most transitory starch was mobilized and translocated to grains during about a month after heading. After that, starch was considerably reaccumulated in the basal internodes but not in leaf-sheaths (Fig. 2, 3).[4,10]

Before heading, nitrogen content of shoots showed, in general, a negative correlation with starch content, probably because more carbohydrates were consumed by more growth and more respiration of plants with higher nitrogen content than plants of lower nitrogen content, resulting in less starch remaining in the former.

Changes of starch granules associated with tissue development

The initials of shoot apex seem to contain no amyloplast. Corpus cells of two to three layers beneath the initials and cells of slightly

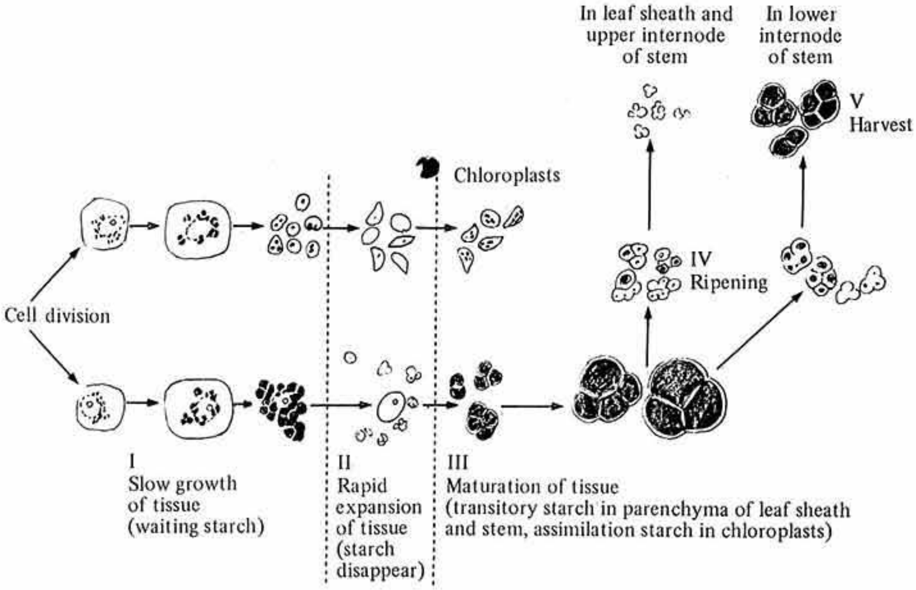

Fig. 4. Transformation of plastids and starch granules (stained with IKI) during the course of tissue growth and kernel ripening

elongated leaf primordia began to contain small amyloplasts with small compound starch granules which become larger with gradual cell expansion.[1,9] As shown in Fig. 4, these starch granules were consumed by the rapid expansion of the cells containing them, and disappeared. These starch granules are distinguished from the so-called "transitory starch" which is destined to be mobilized and translocated to other tissues before consumption.[1]

The initials of root apex and periblem cells contained minute plastids with starch granules and a trace of lamella structure.[9] Very young meta-vessel cells near the root apex showed larger amyloplasts than other plerome cells, and these starch granules also disappeared during rapid expansion of the cells. Tiny and larger amyloplasts exist in calyptrogen and matured root cap, respectively, and the latter functions as "statolith."[1,9]

The largest starch granules are accumulated when the largest amount is deposited in the leaf-sheath parenchyma, showing a strong correlation of granule size with starch content. Similarly, in the internode, the largest granules accumulate at the time when the kernel ripening begins (Fig. 3). During the ripening, starch granules in leaf-sheath diminish in size and number and disappear when the leaf becomes old and dies. The starch granules in internode also diminish their size and number with progress of ripening, but after about one month of ripening they again appear in the lower internodes, because at this time kernel ripening almost ceases but photosynthesis is still acting (Fig. 3, 4).[1,4,10]

As starch granules grow bigger with aging of the cell containing them, older or lower internodes and upper portion of each internode contain more and larger starch granules, while the portion of internodes which has just expanded contains no starch. The similar relation exists with leaf-sheaths. Therefore, when a transverse section of the basal part of a rice shoot at vegetative stage is stained with I-IK solution, both outside and inside leaf-sheaths stain black with a no-stained sheath which has ceased rapid expansion between them. The outside sheaths contain transitory starch and the inside sheaths waiting starch.[1] In the other organs and tissues, similar relations exist. Moreover, size of starch granules varies with varieties and environmental fatcors.

Accumulation of starch in kernels

Most of the kernel starch directly depends on photosynthesis after heading. The contribution of the transitory starch stored in shoots before heading to kernel growth was variable, depending on varieties, environments and management practices. For instance, when the plant was grown under nitrogen deficiency before heading, the contribution rate of transitory starch was greater than that of the plant to which much nitrogen was applied.

Just after fertilization, compound starch granules similar in appearance to transitory starch accumulated in mesocarp parenchyma, and grew in size reaching 15–20 μ after 7 days, but then decreased in amount and disappeared after two weeks probably mobilized and translocated into endosperm.[5] In the endosperm rapid nucleus divisions occur and cell wall is produced simultaneously around each nucleus 4–5 days after fertilization. At this time, minute compound starch granules of a special shape emerged around the nucleus, and grew in size reaching 15 μ and 25 μ one week and two weeks respectively after fertilization.[5] The period to reach the largest size of starch granules depends on varieties and environments, especially on temperature (unpublished data). The period to reach maturity became shorter, but the kernel size smaller with increase of temperature.[8]

Nucellus and outer integument cells began to be collapsed and absorbed 3–4 days after fertilization. On the 5th day, most of the nucellus cells disappeared except the nucellar projection which is connected with the dorsiventral vascular bundle of the ovary and the nucellar epidermis. Both of them persist dur-

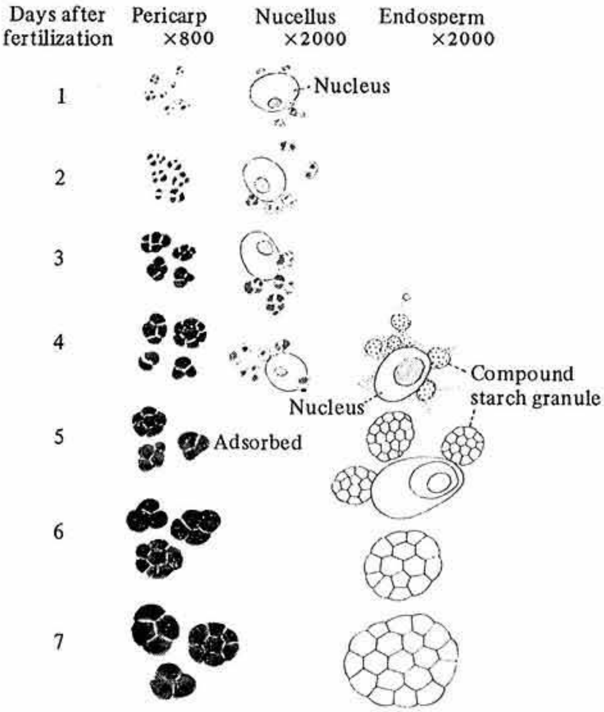

Fig. 5. Development of starch granules at pericarp, nucellus and endosperm during 7 days after fertilization

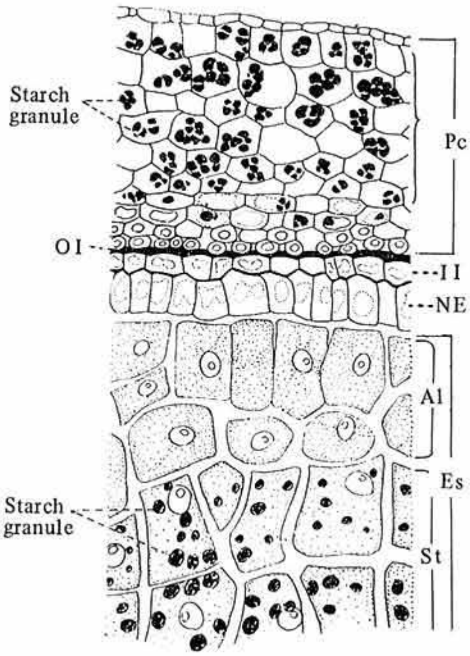

Fig. 6. Longitudinal section of the ventral side of an ovary 7 days after anthesis
Pc: Pericarp
OI: Outer integument
II: Inner integument
NE: Nucellus epidermis
Es: Endosperm
Al: Aleurone layer
St: Starch layer

ing the ripening period[5] (Fig. 5, 6).

From anatomical observations two pathways of nutrients into kernels were postulated; nutrients which come up through dorsiventral vascular bundle go into endosperm as follows[5]:

1) dorsiventral vascular bundle → nucellar projection → endosperm
2) dorsiventral vascular bundle → nucellar epidermis → endosperm

Classification of starch

As already mentioned, young cells near meristems contain relatively small starch granules which may be formed of carbohydrates translocated directly from leaves or indirectly mobilized from transitory starch stored in other tissues. During the active multiplication of the cells, sugars translocated into the cells are fully consumed to produce new cells, leaving no surplus. After the active cell division ends, the cells grow slowly, leaving surplus of sugars, which is deposited as starch granules. These starch granules are exhausted during the following stage of rapid expansion of the cells. These starch granules are named "waiting starch" by the author. This starch exists for a short period before it is used up in the cells themselves.

After the rapid expansion, differentiation of the cells occurs. In the expanded parenchymatous cells of leaf-sheath and internode, sometimes of leaf-blade and roots, too, starch accumulation begins in plastids. The starch granules are larger in size and of longer duration than waiting starch. This starch is named "transitory starch", because it will be mobilized and translocated to other tissues to be consumed there.

Rice leaves are sugar leaves and deposit in chloroplasts only minute single or compound starch granules commonly called "assimilation starch", which is of very short duration. On the other hand, endosperm starch commonly called "storage starch" should better be called "permanent storage starch", because it persists for a long period until seed germination.

Waiting starch is in the form of compound granule composed of several units, and its size reaches 3–4 μ at the most. Transitory starch shows compound granule composed of more number of units with larger size, reaching 20 μ. Physical and chemical nature of these two starches may be similar. Granules of permanent storage starch are composed of around 20 units at surface view, and its size reaches 30–40 μ at the most.

These starch granules differed not only in shape and size, but also in color reaction to iodine. For example, in glutinous rice, glutinous starch which shows reddish purple color with I-KI exists only in pollen and endosperm, but not in all other tissues. This phenomenon exists similarly in x, 2x, 3x and 4x plants of glutinous rice. Culm starch (starch contained in leaf-sheath and stem) differed from endosperm starch in several points. Alkali decomposition values of culm starch were greater than those of endosperm starch and varied among polyploids. The glutinous endosperm starch of glutinous varieties showed a stronger resistance to alkali than non-glutinous endosperm starch.

Absorption curve of iodine-starch complex of culm or endosperm starch varies more or less among varieties and polyploids. Peak of the absorption curve of culm starch was at 600 to 610 nm, while that of endosperm starch at shorter wavelengths than culm starch. Absorption curves of endosperm starch in kernels ripened at different air-temperatures were almost similar, but that of kernels ripened at 35°/30°C (day/night) showed a peak at a shorter wavelength than that at 25°/20°C.

X-ray diffraction pattern of endosperm starch was A-type, while that of culm starch B-type. It suggests differences in polymerisation and chain-length of molecules between the two. No clear difference was observed in X-ray diffraction pattern among polyploids and varieties.

Location of these types of starch in rice plants is as follows:

Type	*Location*
1. Temporary starch	
1) Assimilation starch	Chloroplast
2) Waiting starch	Young cells before rapid expansion, which are located near meristems
3) Transitory starch	Parenchymatous cells of stem (node, internode, rachis and its branch, rachilla), leaf-sheath, leaf-blade, root cap, root, pericarp, etc.
2. Permanent starch	
4) Permanent storage starch	Endosperm

These types differed each other in degree of crystalisation and duration. The lower in the above table, the greater the degree. In general, when the amount of transitory starch at parenchymatous cell is abundant, that of waiting starch near meristems is also abundant. However, even when transitory starch completely disappears, in such a case as grown under shading for a long time, waiting starch is often preferentially preserved near meristems. The transitory starch stored at the basal internodes at harvest was used for the next ratoon crop.

Distribution of photosynthetic tissues and starch-storing tissues in a whole plant at the end of vegetative growth stage

The majority of the leaf-blade is occupied by photosynthetic chlorenchyma, while that of leaf-sheath is starch-storing parenchyma. In a given leaf-blade, peripheral chlorenchyma produces more photosynthate and stores less assimilation starch than central chlorenchyma, and vascular bundle sheath cells usually contain amyloplasts which accumulate transitory starch granules. The similar trend is seen in leaf-sheaths, although production of photosynthates is far less and their storage is far more than leaf-blade.

On the other hand, stems show the highest ability to store starch, although it carries out photosynthesis, and the lower internodes accumulate more starch than upper internodes of the stem. In a given internode, more starch is accumulated in the inner portion of the internode. Similar to stems, leaf-sheaths accumulate more starch in their lower portion.

Therefore, it can be said in short that the upper and peripheral portions of a rice plant produce more photosynthate and store less starch than the lower and inner portions. This relationship is expressed as shown in Fig. 7.

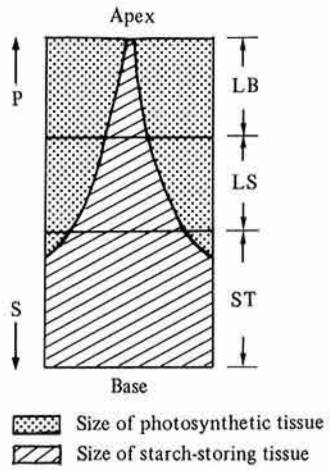

Fig. 7. Diagramatic expression of distribution of two different tissues, photosynthetic tissue (P) and starch-storing tissue (S) in a rice shoot at the heading stage
LB: Leaf blade
LS: Leaf sheath
ST: Stem

Physiological significance of tissue starch

In general, when sugar consumption for growth exceeds photosynthetic production of sugars, such as in the case of low light intensity or heavy nitrogen application, starch content in plants becomes low (Table 1).

However, the starch content in plants is not the only factor determining grain yields.

Table 1. Effects of nitrogen application and shading on organic constituents (% on dry weight basis) of vegetative rice plants

(Unpublished data)

Treatment	Organ	Dry matter[3] g	Total sugar %	Crude starch %	Soluble nitrogen %	Protein nitrogen %
Much nitrogen	L B[1]	5.73	2.52	1.08	0.40	5.28
	LS+ST[2]	6.00	1.75	6.07	0.80	2.49
	Roots	2.55	2.05	3.31	0.80	2.15
Less nitrogen	L B	2.30	4.42	1.66	0.40	2.76
	LS+ST	3.80	3.22	22.34	0.40	0.82
	Roots	3.30	4.33	2.74	0.62	0.72
Shading	L B	1.15	1.00	0.58	0.63	4.01
	LS+ST	1.10	1.13	2.66	1.25	2.36
	Roots	0.85	1.00	2.39	0.83	2.16

1) Leaf-blade, 2) Leaf-sheath and stem, 3) Total dry weight per 5 plants

For example, plants grown under nitrogen deficiency show a higher content of transitory starch, but their yield is low due to their small size and low photosynthetic ability during ripening. On the contrary, plants grown at a high nitrogen level show a low content of transitory starch, but their shoots are larger and have a greater ability of photosynthesis due to higher nitrogen content in leaves.[6]

As shown above, the transitory starch is accumulated in culms and leaf-sheaths due to decreased growth rate at the late stage of vegetative growth, during which photosynthesis still continues at a high level. This starch is mobilized and translocated into kernels after heading. In addition, this starch can be utilized as a material for recovering from various damages caused by diseases and insect pests, flooding, low temperature, or defoliation.[6,7] Furthermore, it gives a resistance to culms against lodging.[2]

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