Keynote Speech

Accumulation Structure of Storage Substances in Organs of High-Starch-Yielding Crops

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Abstract
Humans have selected crops with high starch productivity, created new cultivars, and developed high yielding and quality cultivations in the history of agriculture. Starch productivity and accumulation features of storage starch differ among crop species and its cultivars. This paper describes morphological and anatomical analyses of accumulation structure of storage substances in organs, using a scanning electron microscope. In order to observe storage substances in tissues and cells of plant body by scanning electron microscope with high resolution, complete dehydration of specimen is necessary after fixing. This paper also introduces 'rapid freezing-high vacuum freeze drying method' to prevent artifacts. Starch accumulation density is higher in corn (maize) endosperm and rice endosperm, which is considered to contribute to high starch productivity. Potato tuber, sago palm stem and wheat endosperm have been also utilized by their specific ingredients and shapes or forms in their accumulation structure. In the future, demand for starch in Japan and the world will further be expanded. In future not only "quantity" of starch, but also "quality" and "palatability" of starch will be considered.

Keywords: Accumulation, Amyloplast, Electron microscope, Protein, Starch

Introduction

In crop organs, feature of accumulation structure of storage substances such as starch, protein, lipid, etc. are so diverse among crop species as well as cultivars. Starch, because it is produced in a chloroplast by photosynthesis activity, is also called an assimilation starch. The assimilation starch is converted into soluble sugar, and transported into storage organs such as roots, stems, seeds, etc., followed by resynthesized of starch in plastid which is a kind of cell organelle. Then, in a plastid, after lot of starch accumulation, it becomes an amyloplast which is also a kind of cell organelle. Finally, starch is accumulated in an amyloplast.

In the history of agriculture, humans have selected crops with high starch productivity, created new cultivars, and conducted high yield and quality cultivation with improving management techniques. Starch productivity and accumulation features of storage starch vary depending on crop species and its cultivars.

This paper describes morphological and anatomical accumulation structure of storage substances of high-starch productivity crops, which are grown in the tropical, sub-tropical and temperate regions in organs, by using a scanning electron microscope.

Materials and methods

1. Specimen preparation for observation of storage substances by scanning electron microscope

In order to observe storage substances in tissues and cells of plant body by scanning electron microscope with high resolution, complete dehydration of specimen is necessary after fixing. However, in tissues and cells of a plant body, during fixing and/or dehydration process, changes of their original structure would occur often, unexpected structures and/or substances would be produced as well. The latter one is called artifact. Prevention of the production of artifact and unexpected structures, and keeping their original structure during fixing and/or dehydration process is an important factor for observation by scanning electron microscope.

In the author's laboratory, 'rapid freezing-high vacuum freeze drying method' (Matsuda, 2003), which is a kind of physical fixing and freeze-drying method (Kawasaki and Matsuda, 2006), is commonly used for prevention of produce artifact and keeping their original structures. Outline of its procedure is as follows.

- Store and submerge plant raw materials in fixing solution sufficiently.
- Submerge materials in water and dissect it to small volumes (ex. 5 mm length × 5 mm width

× 2 mm thickness).

- Small species of material is put into slush nitrogen (-210 °C) for rapid freezing, followed by vacuum freeze drying (-0 °C, 10-3 Pa).
- Completely dried up species is mounted on stub.
- Surface and revealed cross section is coated by OsO_4 and/or platinum.
- Observe by scanning electron microscope (ex. JSM6360A; JEOL, Japan).
- Lengths, sizes and areas of tissues and cells are measured using specific image analyzing software (ex. WinROOF; Mitani Co. Ltd., Japan)

Results and Discussion

2. Accumulation structure of storage substances in organs of high-starch-yielding crops

Many crops produce and accumulate starches. Figure 1 shows an example of accumulation structure of storage substances of several crops (Scale: 10 µm).

Among of storage substances, starch storage organs differ among crops: (A) grains are used by rice, wheat, corn (maize), barley, and other cereal crops; (B) tubers are used by potato, Jerusalem artichokes, and arrowhead; (C) tuberous root are used by sweet potato, cassava, dahlia, and snake gourd plants; and (D) other organs are used by for lotus, and banana plants.

Regarding starch accumulation in an amyloplast, if starch grains accumulate there, they are called a 'simple starch grain' such as sago palm stems, potato tubers, and yam tubers. If multiple starch grains accumulate in an amyloplast, the starch accumulation is called a 'compound starch grain' such as rice grains, taro tubers, and sweet potato tuberous roots.

For cereal crops, accumulation features of storage substances such as starches, proteins, and lipids are investigated intensively, suggesting that starch accumulation features are widely diverse among species, cultivars, and varieties as well as growing area, culturing methods, and environmental conditions.

In parenchyma cell of potato tuber, shape of amyloplast is like a crushed boiled egg. Small amyloplast is produced after enlargement of each amyloplast, so that the sizes of amyloplasts are so diverse (longer axis: 10 – 100 µm).

Shape of amyloplast of yam tuber is like a steamed bun with triangle to pentagon form. Difference of the size of amyloplasts is small, taking around 15 to 30 µm in longer axis.

Amyloplast of sweet potato tuberous root takes shape like a rice ball with 10 to 50 µm in longer axis. Each amyloplast contains around several to 20 starch granules. Shape of starch granule is tetrahedron to polyhedron with 3 to 10 µm in longer axis.

Shape of amyloplast of taro tuber is ellipsoid with bumpy surface taking around 10 to 20 µm in longer axis. Longer axis of starch granule is so small (only 0.5 to 2.0 µm), so that it is a causal factor for smoothness when we take it in mouth. There are around 100 to 4000 starch granules in an amyloplast.

In a parenchyma cell of sago palm stem, amyloplasts with an egg like shape accumulate densely. However, it is specific character for sago palm stem that intercellular spaces are so large. Percentage of intercellular spaces takes around 40 to 50% in cultivars and stem positions.

In an endosperm of wheat grain, there are two types of amyloplast: primary starch grain (amyloplast) with around 20 to 40 µm in longer axis, and secondary starch grain (amyloplast) with around 2 to 8 µm in loger axis. In addition, protein granules and protein matrix are filled around starch granules densely.

Longer axis of an amyloplast of corn (maize) endosperm takes around 2 to 10 µm. In hard starch tissues of endosperm of flint corn (only hard starch tissue) and dent corn, amyloplasts accumulate densely without spaces.

In a rice endosperm, amyloplast with around 10 to 15 µm in longer axis contain around several to 100 starch granules with 3 to 5 µm in longer axis. In case of nonglutinous (nonwaxy) rice cultivars, accumulation structure is so precise without any spaces among amyloplasts. While in brewer's rice cultivars, dent portions on amyloplast surface and hollow spaces among amyloplasts cause chalky structure in their appearance.

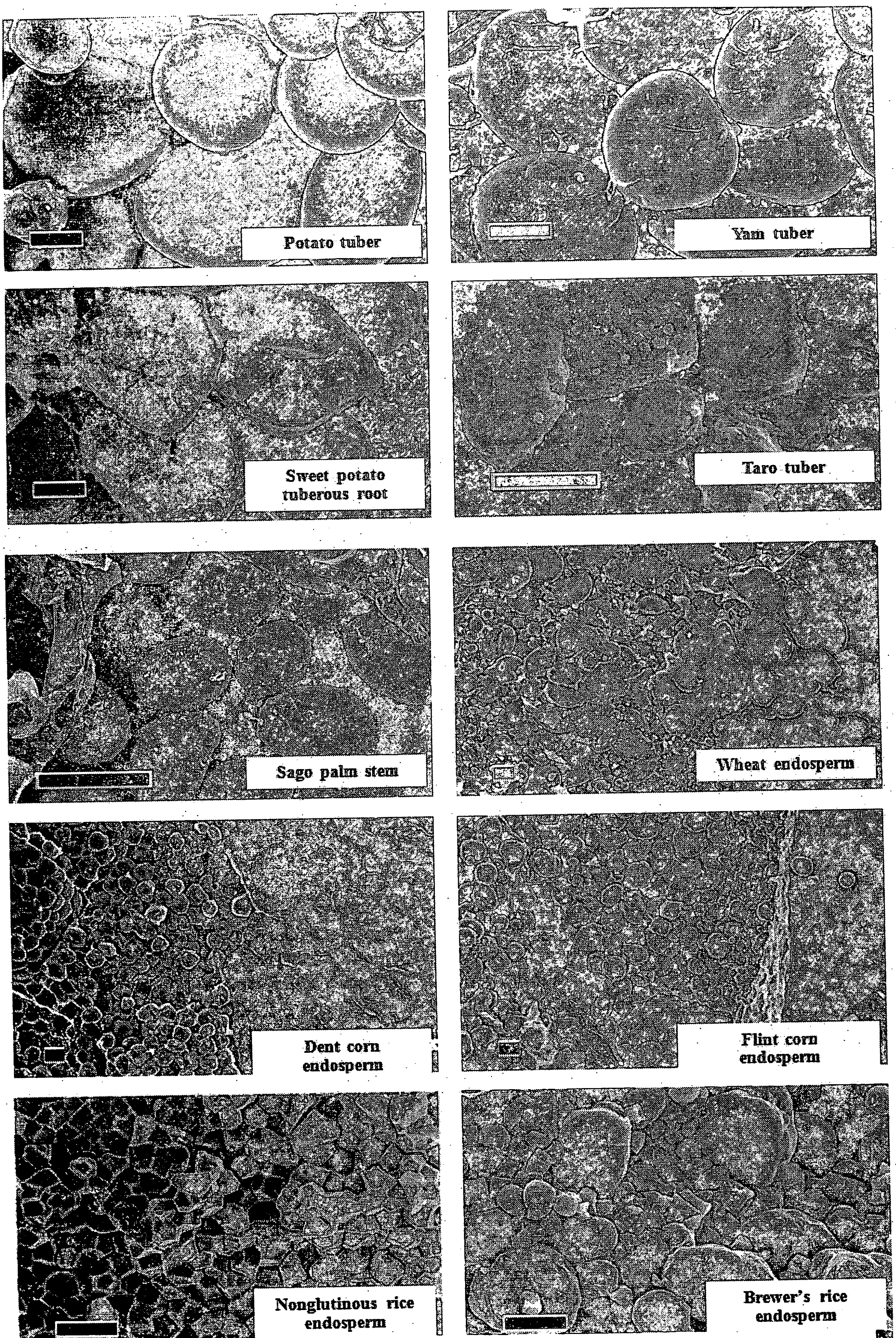

Figure 1: accumulation structure of storage substances of several crops (Scale: 10 µm)

Conclusion

Starch can be produced only by higher plants and accumulated in seeds, roots, stems and other organs. In grains of cereals such as rice, wheat and corn (maize), starch account for more than 70% of ingredients, provide important food for many people. Recent years, starch has also been used as a bio-fuel material which produce bio-ethanol after saccharification and fermentation. Usage of starch for biofuel is increasing. Among the high-starch productive crops studied in this experiment, accumulation density of starch is higher in corn (maize) endosperm and rice endosperm, which is considered to contribute to high starch productivity. Moreover, starch accumulation structure of potato tuber, sago palm stem and wheat endosperm differ in their specific ingredients and shapes or forms. In future, demand for starch in Japan and the world will further be expanded. In addition, in future not only requirement for "quantity" of starch, but also "quality" and "palatability" of starch will be important.

Reference

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