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Morphological Studies on the Mobilization of Reserves in Japanese Yam (*Dioscorea japonica* Thunb.) Seed Tuber and Eddo (*Colocasia esculenta* Schott var. *antiquorum* Hubbard & Rehder) Seed Corm on and after Sprouting

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Abstract : We examined the mechanism of reserve mobilization in Japanese yam seed tubers and eddo seed corms on and after sprouting. The decomposition of starch in pith parenchyma cells of Japanese yam tubers and eddo corms progressed from the region distant from vascular bundles to that adjacent to vascular bundles. In eddo corms, the starch also decomposed from the proximal to the distal region adjacent to the sprout or regenerate plant body. In the yam tubers, the decomposition process was similar in the proximal, middle and distal regions. The first step in the reserve mobilization in pith parenchyma cells was the decomposition of the amyloplast envelope. Subsequently, starch granules decomposed. In Japanese yam tubers, the envelope and starch granules started to decompose from the peripheral regions of the amyloplasts. The observation of soluble polysaccharides, which was the decomposition product of starch granules, was made possible by the quick freezing-vacuum freeze-drying method. By this method, we demonstrated that the soluble polysaccharides in the parenchyma cells decomposed and decreased in density. In addition, the mucilage in the mucilage duct started to decompose and decreased in density from the proximal to the distal part of the corm and also from the periphery to the center of the duct. It was shown that not only starch mobilization but also mucilage mobilization mainly supported sprouting and the growth of the regenerate plant body during about the first half of the vegetative stage.

Key words : Amyloplast, Arabinogalactan, Decomposition, Electron microscopy, Sprout, Starch, Taro, Yam.

Edible yams and taros are tropical crops that serve as important staple foods in many parts of the world. In Japan, Japanese yam "yamanoimo" (*Dioscorea japonica* Thunb.) belonging to the group of yams and eddo "satoimo" (*Colocasia esculenta* Schott var. *antiquorum* Hubbard & Rehder) belonging to the group of taros have been eaten since early times. Yam tubers and eddo corms contain plenty of starch. The starch content is 704 ~800 g kg^{-1} (dry weight basis) in the former (Agbor-Egbe and Treche, 1995; Hariprakash and Nambisan, 1996) and 509~662 g kg^{-1} in the latter (Agbor-Egbe and Rickard, 1990). In eddo corms, appreciable quantities of mucilage are also present. The yield of mucilage from 12 taro varieties varied from 76 to 137 g kg^{-1} (Gaosong and Lawrence, 1999). Analysis of the mucilage revealed galactose and arabinose as the main monosaccharides and arabinogalactan-protein as the main polymer (Gaosong and Lawrence, 1999). A histochemical study identified arabinogalactan-protein in the mucilage ducts that were scattered throughout the corm (Harris et al., 1992). However, the precise functions of arabinogalactan-proteins in plants (Majewska-Sawka and Nothnagel, 2000) and the mucilage itself in eddo corms have not been clarified yet.

Many investigators have studied the chemical composition of yam tubers (Ravindran and Wanasundera, 1992 ; Agbor-Egbe and Treche, 1995; Hariprakash and Nambisan, 1996; Omonigho and Ikenebomeh, 2000) and taro corms (Agbor-Egbe and Rickard, 1990; Gaosong and Lawrence, 1999) during storage. However, a few reports have been made related to the chemical composition of the tubers and corms after sprouting (Hariprakash and Nambisan, 1996). Several investigators have studied the morphological change with respect to the reserve mobilization in rice seeds (Zakaria and Matsuda, 1999; Zakaria et al., 2000), soybeans (Kashiwaba et al., 1995) and commom beans (Kashiwaba et al., 1998) on and after germination. However, little is known about the reserve mobilization in storage vegetative organs on and after sprouting, especially in yam tubers and taro corms. In addition, to our knowledge, there are no reports dealing with the fate of soluble substances such as arabinogalactan-protein.

Yam tubers and taro corms have a natural dormancy

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Abbreviations : A, amyloplast; MD, mucilage duct; SG, starch granules; VE, vascular bundle.

period, after which they sprout and regenerate plant bodies. Much reserve is used for growth of regenerate plant bodies from sprouting to the middle vegetative stage in eddo corm and from sprouting to the end of July in Japanese yam tuber (Hoshikawa, 1980). Eddo grows slowly during early period of vegetative growth (Hoshikawa, 1980). Therefore, the acceleration of the reserve mobilization and its efficient use are necessary for promoting for the growth and increase of yield. The present study was done to elucidate the mechanism of reserve mobilization on and after sprouting in Japanese yam seed tubers and in eddo seed corms. We examined the ultrastructural changes of the starch and the mucilage on and after sprouting by scanning electron microscopy and discussed the function of the mucilage in eddo seed corms, using the samples fixed by quick freezing-vacuum freeze-drying method (Matsuda et al., 1995b).

Materials and Methods

1. Plant materials

The seed tuber of Japanese yam "yamanoimo" (*Dioscorea japonica* Thunb.) and the seed corm of eddoe "satoimo" (*Colocasia esculenta* Schott var. *antiquorum* Hubbard & Rehder) variety "Dotare" were used in this experiment. Both materials were planted in the experimental fields of Ibaraki University at Ami, Japan, in June 1996. The planted seed tubers and corms were sampled 3 days before sprouting, on the day of sprouting, 7th day and every 14th day after sprouting for scanning electron microscopy.

In this paper, "sprouting" is defined as the appearance of sprouts.

2. Scanning electron microscopy

The pith tissues, which store most of the reserves in Japanese yam tuber (Kawasaki et al., 1997) and eddo corm (Kawasaki et al., 1998), were fixed with the quick freezing-vacuum freeze-drying method for scanning electron microscopy. This method is suited for the observation of soluble polysaccharides (Matsuda et al., 1995b). Soluble polysaccharides do not flow out and are not powdered by this method although they flow out by osmium-dimethyl sulfoxide-osmium method (O-D-O method) (Tanaka et al., 1981; Matsuda et al., 1995a) and are powdered by usual vacuum freeze-drying methods. The small blocks from the pith tissue were obtained with a razor blade. The blocks were frozen rapidly with slush nitrogen (−210°C), followed by vacuum freeze-drying (OTD-5SF-S; Oka Science, −60°C, 10^{-3} Pa). The dried blocks were cut transversely with a razor blade. The cut blocks were attached to the specimen stubs, and coated with platinum so that the cut surface was exposed. The specimens were viewed with a scanning electron microscope at an accelerating voltage of 5 kV (JSM6301F).

Results

1. The mobilization of starch in Japanese yam seed tubers on and after sprouting

Fig. 1 shows the pith parenchyma tissue of Japanese yam tuber at the 28th day after sprouting when the vine length of the regenerate plant body is 80～120 cm in length. The starch granules in pith parenchyma cells started decompose from the region distant from the vascular bundles toward vascular bundles. However, it was uncertain whether the starch decomposition began from the proximal or distal region of the seed tuber.

The mobilization pattern of starch in pith parenchyma cells was as follows. Before sprouting, there were many amyloplasts, 15～25 μm in diameter, that were enveloped in intact envelope membranes in the pith parenchyma cells (Fig. 2). On these amyloplasts, however, a "projection" formed on the amyloplast during thickening growth (Kawasaki et al., 1997) was not observed (Fig. 2).

The first step in the mobilization of reserves in the pith parenchyma cells on and after sprouting was the decomposition of amyloplast envelopes. Fig.3 shows the amyloplast envelopes (*) which were not decomposed in the nonperipheral region of the amyloplast but had already been decomposed at the peripheral region. In addition, sol-like substance was observed abundantly near the periphery of an amyloplast as shown by arrows in Fig. 3. With the growth of the regenerate plant body, the starch granules gradually changed to sol-like substance (Figs. 4, 5 and 6). Later, the sol-like substance had changed to show a reticular structure and decreased constructing the expansion of its lattice-like structure (Fig. 6).

The relationship between the mobilization phase of storage starch in pith parenchyma cells and the time after sprouting was not clear, because various mobilization phases were observed at each developmental stage of the tuber. However, most of the starch had disappeard from many pith parenchyma cells at about 98 days after sprouting when the vine length was over 200 cm.

2. The mobilization of starch and mucilage in eddo seed corms on and after sprouting

In eddo corms, the decomposition of starch in the pith parenchyma cells started from the region distant from vascular bundles toward the vascular bundles, and also from the proximal to distal region adjacent to the sprout or regenerate plant body. Exceptionally, starch granules in the parenchyma cells around the mucilage ducts had been decomposed more slowly than in other cells at a distance from vascular bundles (Fig. 12).

The mobilization of starch in a pith parenchyma cell was as follows. There were many amyloplasts about 15～35 μm in diameter enveloped in an intact envelope membrane in pith parenchyma cells before sprouting (Fig. 8). From sprouting onward, the decomposition of amyloplast envelope membrans was observed first in

eddo tuber (Fig. 9). After that, very small starch granules in it gradually changed to sol-like substance (arrows in Figs. 9 and 10). After showing a reticular structure, the sol-like substance decreased constructing lattice-like structure (Fig. 11).

The mucilage (*) in mucilage ducts started to decompose from the proximal to distal region of seed corms from about 7～14 days after sprouting when the main corms were in 1～2 leaves (Fig. 12). The mobilization of mucilage in a mucilage duct was as follows. Fig. 12 shows the dense mucilage in mucilage ducts 7 days after sprouting. With growth of regenerate plant bodies, the mucilage decomposed and changing to low-density material gradually from the peripheral region of the duct as shown by an arrow in Fig. 13. Fig. 14 shows the mucilage shown as parallel structure during the decomposition of mucilage in the duct. With growing of the plant body, most of the mucilage had decomposed (Fig. 15).

The relationship between the phase of reserve mobilization in pith parenchyma cells and the time after sprouting was not clear in eddo corm, either. However, starch granules were scarcely observed in many pith parenchyma cells at about 74 days after sprouting and most of the mucilage in many mucilage ducts disappeared at about 88 days after sprouting, when the main corm was in 5～7 leaves.

Discussion

Starch in pith parenchyma cells of Japanese yam tubers and eddo corms decomposed gradually on and after sprouting from the region distant from vascular bundles towards the vascular bundles. In the stem of potato, starch granules decomposed from the region distant from the phloem toward the phloem during the vegetative stage (Yoshida, 1970). Giaquinta (1983) reported that sucrose in the mesophyll cells was transferred from the part distant from the phloem toward phloem via symplast and then into the phloem from the mesophyll cells adjacent to the phloem via apoplast. Therefore, the decomposition products of starch granules such as sucrose in pith parenchyma cells of the tubers and the corms would be transferred from the region distant from the phloem toward the phloem via symplast. In eddo, the starch decomposed from the proximal to the distal parts of the corm. Yoshida (1970) reported that starch granules decomposed from the proximal to the middle part of stem in potato at the late vegetative stage. In rice seed, however, starch granules were degraded from the region adjacent to the scutellar epithelium to the distal region (Zakaria et al., 2000). In Japanese yam tubers, it is uncertain whether the starch started to decompose from the proximal or distal region of the seed tuber. According to Hariprakash and Nambisan (1996), the rates of decrease of starch contents in the proximal, middle and distal regions of tubers without planting for 60～70 days after sprouting were 33, 29 and 41%, respectively, in *Dioscorea rotundata*. The rates were only slightly different in *Discorea esculenta*. In Japanese yam tubers, starch in the proximal, middle and distal

Explanation of figures

Figs. 1～7 show scanning electron micrographs related to the decomposition of starch in Japanese yam seed tubers. (Fig. 1; Bar = 100 μm) (Fig. 2～7; Bars = 10 μm)

Fig. 1. Pith parenchyma tissue in a tuber at the 28th day after sprouting.

Figs. 2～7 show the decomposition process of starch sequentially.

Fig. 2. Amyloplasts in a pith parenchyma cell before sprouting.

Fig. 3. The decomposition of amyloplast envelopes (*) in the pith parenchyma cell. Starch granules decompose from the peripheral part of amyloplasts after sprouting. Sol-like substance (arrows) at the periphery of amyloplast.

Figs. 4 and 5. Starch granules decomposing to sol-like substance (arrows) in a pith parenchyma cell.

Fig. 6. The disappearance of starch granules and the increase in sol-like substance (arrows) in the parenchyma cell.

Fig. 7. Sol-like substance (arrows) showing reticular structure decreases and expands to show lattice-like pattern.

Figs. 8～11 show scanning electron micrographs related to the decomposition of starch in eddo seed corm sequentially. (Figs. 8, 10 and 11; Bars = 10 μm) (Fig. 9; Bar = 1 μm).

Fig. 8. Amyloplasts in a pith parenchyma cell before sprouting.

Fig. 9. Starch granules decomposing after the decomposition of amyloplast envelope and changing to sol-like substance (an arrow).

Fig. 10. Starch granules changing to sol-like substance (arrows) in a pith parenchyma cell.

Fig. 11. Sol-like substance (arrows) showing reticular structure and decreases and expands to show lattice-like pattern in parenchyma cell.

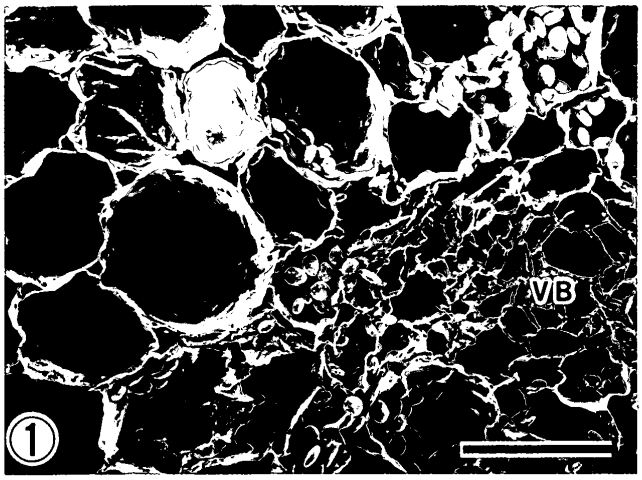
Figs. 12～15 show scanning electron micrographs related to the decomposition of the mucilage in mucilage ducts of eddo seed corms sequentially. (Figs. 12, 13 and 15; Bars = 100 μm) (Fig. 14; Bar = 1 μm).

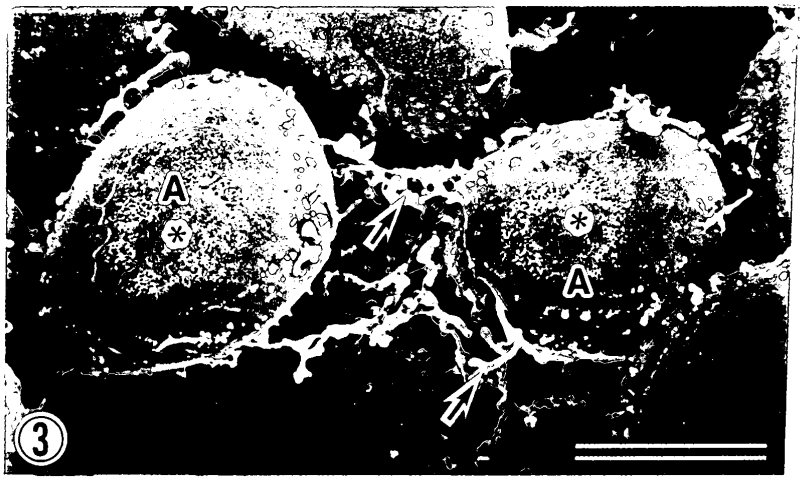
Fig. 12. The mucilage (*) in a mucilage duct 7 days after sprouting.

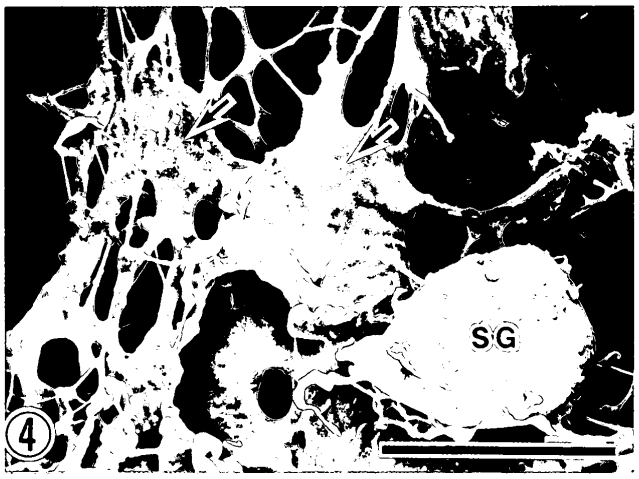
Fig. 13. The mucilage (*) decomposing to show a lower density structure from the periphery (an arrow) in a duct.

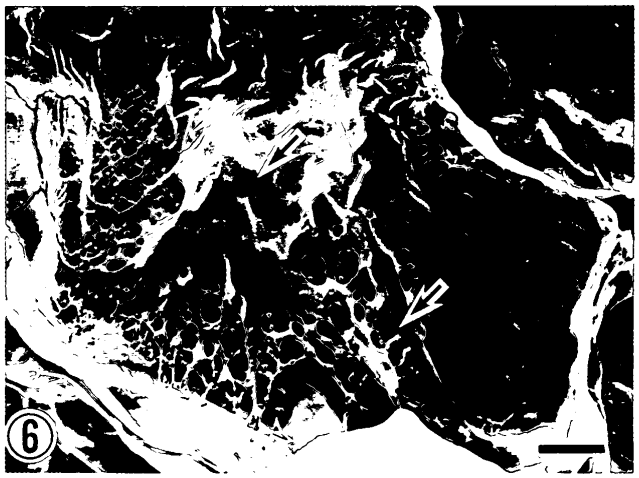
Fig. 14. The mucilage (*) showing parallel structure during decomposition in a duct.

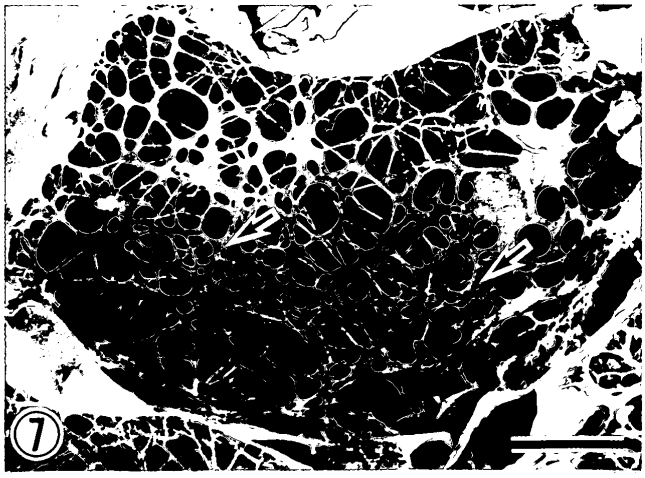
Fig. 15. A mucilage duct with most of mucilage decomposed.

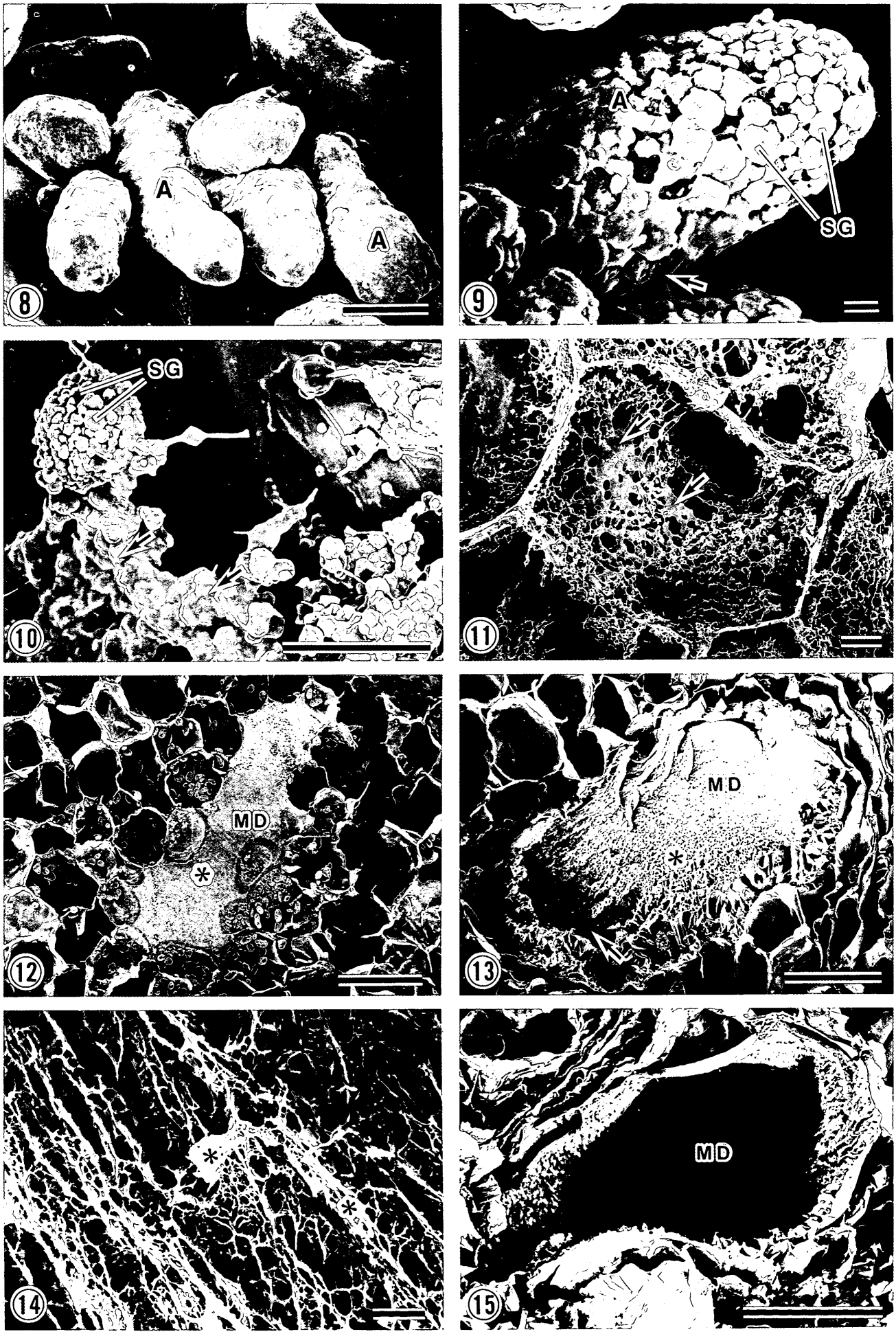












might also be decomposed similarly.

The amyloplasts in the tubers and the corms were enveloped in an envelope membrane before sprouting as at harvest (Kawasaki et al., 1997,1998), but were smaller than at harvest (Kawasaki et al., 1997,1998). Wetzstein and Sterling (1978) reported that the amyloplast envelopes were not broken in potato tuber during storage at 4 and 20°C. Yada et al. (1990) reported that the sugar content in the parenchyma cells of potato tuber increased during storage at 5 and 10°C so that the starch decomposed in the amyloplast with an intact envelope. In stored yam (*Dioscorea esculenta, Dioscorea rotundata, Dioscorea alata*) tubers, the starch content decreased during dormancy (Hariprakash and Nambisan, 1996), suggesting that the starch granules decomposed in amyloplasts with intact envelopes. However, the starch granules gradually changed to sol-like structure after decomposition of the amyloplast envelope in the present observation. This suggests that the mechanism of starch decomposition on and after sprouting in Japanese yam tuber and eddo corm differs from that during dormancy.

Little is known how the amyloplast envelope membrane is decomposed in vivo by morphological study. We showed that the envelope membrane started to decompose from the peripheral region of amyloplasts in Japanese yam tuber. This decomposition might be caused by the physical fragility. However, there is a possibility that it is caused by the action of enzymes in the amyloplast. The stroma, which contain many kinds of enzymes exist, localized mainly in the "projection" and also in other peripheral regions of developing amyloplasts in Japanese yam tuber during thickening growth (Kawasaki et al., 1997). Therefore, the enzymes in the stroma between starch granules and envelopes could act after sprouting.

In this study, we observed soluble polysaccharides, which were produced by the decomposition of starch granules, by the quick freezing-vacuum freeze-drying method. The decomposition product of starch granules abounded near the peripheral region of amyloplasts in our observation. This suggests that starch granules also started to decompose from the peripheral region of amyloplasts in the Japanese yam tuber. In wheat seeds, the peripheral region of starch granules showing an equatorial groove could have access to glucoamylase and α-amylase (Buttrose, 1960). Decomposition would be caused by physical fragility of starch granules, because the peripheral region of the starch granule at the proximity of the "projection" and the "groove" had functioned in starch synthesis until the last stage end of amyoplast development (Buttrose, 1960; Kawasaki et al., 1997) and would be lower in density than the other regions. In addition to the physical fragility of starch granules, debranching enzyme from the amyloplast might also affect the decomposition of the starch granules.

In this study, we demonstrated that the soluble polysaccharides in the parenchyma cell decomposed gradually with the growth of the plant. Furthermore, mucilage in mucilage ducts was found to decompose gradually after sprouting. The mucilage in mucilage ducts (Harris et al., 1992) has been found to contain galactose and arabinose as the main monosaccharides and arabinogalactan (D-galactose : arabinose = 5～6 : 1)-protein as the main polymer (Harris et al., 1992; Gaosong and Lawrence, 1999). Although the mucilage is one of the main components of taro corm, no one has succeeded in identifying the precise functions of arabinogalactan-protein in plants (Majewska-Sawka and Nothnagel, 2000) or of the mucilage itself of taros. However, from our results, it was suggested that the mucilage functioned to supply the substances necessary for growth of the regenerate plant body. Exceptionally, starch granules in the parenchyma cells around the mucilage ducts decomposed slowly, which also suggests that the mucilage ducts supply the decomposed products of mucilage to the parenchyma cells around the ducts. The mucilage showed parallel structure during decomposition in the ducts. A similar structure was also observed in storage polysaccharides in vegetative storage organs of Jerusalem artichoke, tulip, dahlia and others (Matsuda et al., 1995b).

According to Hariprakash and Nambisan (1996), the starch contents in tubers left without planting for 60～70 days after sprouting were 32, 45 and 40% (dry weight basis) of the initial contents in *Dioscorea rotundata, Dioscorea alata* and *Discorea esculenta*, respectively. However, it has been reported that much reserves are used for the growth of regenerate plant body the first half of the vegetative growth in eddo and from sprouting to the end of July in Japanese yam (Hoshikawa, 1980). It was shown that not only starch mobilization but also mucilage mobilization mainly supported sprouting and the growth of the regenerate plant body during about the first half of the vegetative growth.

For better understanding of the mechanism related to the mobilization of reserves, it is necessary to investigate other reserves such as viscous liquid in yam tuber, protein and lipid and to localize the enzymes that breakdown the reserves and the amyloplast membrane, by transmission electron microscopy and immunoelectron microscopy.

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