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MORPHOLOGY AND STRUCTURE OF STARCH GRANULES IN STORAGE ROOT OF PURPLE SWEET POTATO

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Abstract

The microstructure and formation process of starch granule in purple sweet potato tube were examined by light microscopy (LM), transmission electron microscopy (TEM), and scanning electron microscopy (SEM) in this study. The results showed that the proliferation of division activities of amyloplasts was followed by massive accumulation of amyloplasts in cells. The amyloplasts then produced several eccentric starch granules, and the structure of binary and tetrad starch granules was evident upon imaging by LM, TEM, and SEM. In the original formation of starch granule, there were no absolute crystals aggregated. Some blocklet crystals gradually appeared in the starch granules as numerous 'microgranules' were tightly connected into the chain, containing the double helix of amylopectin. Many 'microgranules' were observed on the exterior surface of the starch granule and formed multiple concentric shells which increased the diameter of starch.

Key words: Purple sweet potato, Starch granule, Morphology, Ultrastructure, Development

Introduction

Starch is one of the main foods in our diet, and one of the main energy storage forms in higher plants (Zhang *et al*., 2017). Starch is present in the form of particles and starch granules, which show varying dimensions, size distributions, and shapes in the cells. Comparative studies on the starch granules of maize and potato (Baker *et al*., 2001; Sujka & Jamroz, 2009) showed that potato starch granules are larger than maize starch granules, with a diameter of perhaps l00 μm and is oval in shape (Jenkins *et al.,* 1993). Rice starch granules are small in size (maximum diameter 10 μm) and angular in shape (French, 1984). Plant starch is organized as alternating semi-crystalline, dense lamellae, and amorphous. The semi-crystalline and amorphous layers alternately compose the growth rings, which are broken by dense lamellae crystals. Crystallinity is associated with the branched amylopectin component of the starch granule (Montgomery & Senti, 1958). Some research suggested that young amyloplasts divide by binary fission (Mingo-Castel *et al*., 1991).

Molecular biology tools were used to study the process of starch biosynthesis in depth and the enzymes involved in starch biosynthesis (Ball *et al*., 1996; Ball, 1995). Sucrose from photosynthesis is first degraded into uridine diphosphate glucose, which is then converted to Glucose-6-phosphate (G-6-P) in the presence of pyrophosphate, and fructose by sucrose synthase. Glucose-6-phosphate (G-6-P) is converted to Glucose-1-phosphate (G-1-P) by phosphoglucomutase in the amyloplast, the intracellular organelle responsible for starch biosynthesis in storage tissues. Glucose-1-phosphate (G-1-P) in the amyloplast is transformed into ADP-glucose, providing glucose residues for the biosynthesis of amylose and amylopectin (Denyer *et al*., 2001). Both of amylopectin and amylose are self-assembled with their molecular axes orientated perpendicular to the growth rings (Tang *et al*., 2006). The semicrystalline growth rings include 16 radiant clusters of amylopectin outer chains, the actual length of which is a registered double helix of about 6.65nm (equivalent to crystalline thin slices), scattered in an amorphous thin slice of about 2.2nm (Cameron & Donald, 1992; French, 1984). Most of the products of photosynthesis accumulate in amyloplasts in the form of starch granules.

Some studies on starch structure have been conducted by different methods (Sagisaka, 2008; Wang *et al*., 2008). However, there is a lack of systemic experimental work regarding the ultrastructure of starch granules in the development process of sweetpotato and the relation between starch and anthocyanin in purple sweetpotato. To elucidate the coordinated biological events, microstructures of starch granules were complemented by iodine binding test, scanning electron microscopy (SEM), and transmission electron microscopy (TEM), which were important tools for inner and outer structural studies. This research provides new insights into the ultrastructure of starch and the accumulation process of starch granules and coexistence between starch and anthocyanin, stimulating further research on this topic.

Material and Methods

Plant material: Purple sweetpotatoes seedlings planted (cultivar: King of Purple Sweetpotato) on May 19, 2015 in Linfen, Shanxi province (Longitude: 111.52, Latitude: 36.08); the storage roots of purple sweetpotato were collected after onset of secondary growth in tuberous root (Wilson & Lowe, 1973) because the starch was formed after secondary growth. On October 19, 2015, the samples were collected and classified according to development diameter (10 mm to 200 mm) of storage root. Different classes of purple sweetpotato were divided into 5 mm pieces, formalin acetic acid (FAA) and glutaraldehyde fixing solutions were used to fix the samples.

Light microscopy analysis: Samples were fixed with FAA–alcohol fixative solution for 72 h. The fixed samples were sequentially dehydrated using acetone at

25°C according to methods of Zhang *et al*., (2006), then vitrified with a gradient solution of 100% acetone to 100% xylene, and ultimately embedded in paraffin (Leica, Germany). A microtome (RM 2135, Leica, Germany) and mounted on microscopic slides were used to obtain the 6-8 µm sections. Prior to light microscopy observation, the sections were immediately stained with both of Safranin O and Fast Green FeF or unstained to take advantage of the anthocyanin pigment as a marker. All starch sections were observed using light microscope (50i, Nikon, Japan), and imaged with a video camera (DS-Fi1, Nikon, Japan).

Scanning electron microscopy analysis: Purple sweet potato tubers were quickly rinsed two times in pH 7.0 phosphate buffer (0.1 M) and sequentially dehydrated in acetone. Blocks of 1 mm^3 from tubers were prepared on the sample stage and were coated with gold (10 nm), using a sputter coater after fixing and drying. Starch granules were then observed and photographed with SEM (JSM-7500F, Japan) under high vacuum (Nikolakaki & Christodoulakis, 2007; Zhang *et al*., 2018).

Transmission electron microscopy analysis: Samples were directly fixed in pH 7.0 phosphate buffer (0.1 mol L^{-1}) containing 2% (v/v) glutaraldehyde (Alfa Company, China) at 4°C for 24 h. The samples were then fixed in 1% (w/v) osmium tetroxide solution (SPI Company, USA) for 4 h at 4°C for secondary fixation and dehydrated through a gradient of acetone. Subsequently, the samples were embedded with epoxy resin 812(SPI Company, USA). The embedded samples were cut using a glass knife and Reichert–Jung ultramicrotome to obtain semi-thin sections (1 µm to 2 µm) that were stained with toluidine blue and were examined by light microscopy. Ultrathin sections (60–80 nm) were obtained using a Leica EM UC6 ultramicrotome and a diamond knife to cut the embedded samples, stained with uranyl acetate and lead citrate (Gibbons & Grimstone, 1960). Observation and imaging were conducted using a Hitachi H-7650 transmission electron microscope (TEM).

Results

Microscopic structure of starch granule in purple sweet potato: Starch granule construction is a complex process. Semi-thin section images showed that each cell contained several starch granules, some elliptical and some polygonal. Moreover, binary and tetrad starch granules were obvious in the cells (Fig. 1A), thereby increasing the number of starch granules. Sections were prepared according to the modified paraffin section method (Zhang *et al*., 2006). The results showed that star-like crystals existed in starch granules of the purple sweet potato cells (Fig. 1B). Carotenoids occur as membrane-bound similar crystalline structures in yellow fresh sweet potato (Tumuhimbise *et al*., 2009). Further, we also observed a hole in the centre of some star-like crystals.

Starch granules existed in the cambium and parenchymal cells in the xylem of the tuber (Fig. 1A). Since the starch granules were originally formed in cambium cells, crystals appeared as tiny spots in these cells. Moreover, development of the tuber and increasing number of starch granules resulted in the increase in the size of the crystals and starch granules, accompanied by the gradual transformation of crystals into a needle- or star-shaped crystals.

Analysis of microstructures of starch granules was complemented by the iodine binding test. After the iodine-binding treatment, images of starch granules appeared blue. Furthermore, there were tiny blue points around the starch granule, indicating the presence of oligomers of starch. The images also showed a star-like hole in the centre of the granule (Fig. 1C). Furthermore, the micrograph indicated that irregular distribution of small spherical particles on the surface of starch granules was responsible for the uneven surface of starch.

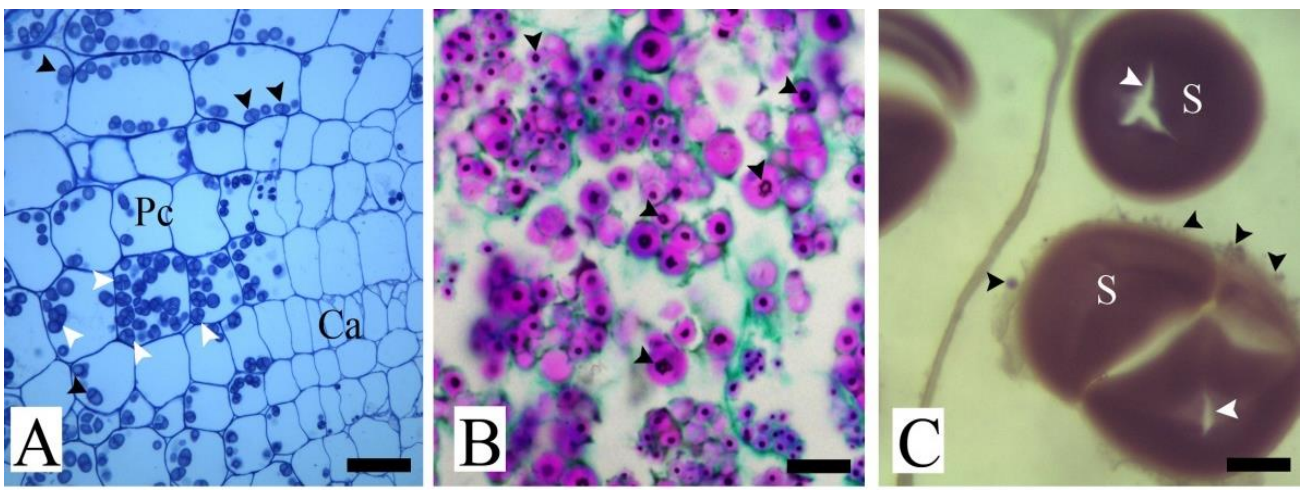

Fig. 1. Microscopic images of a cross-section of purple sweet potato cell. A. Semi-thin section micrographs of starch granule stained with toluidine blue. Black arrows indicate binary structures, while white arrows indicate tetrads. B. Microscopic image of starch granule using improved paraffin section. Black arrows indicate sites of crystals with blank holes. C. Microscopic image of starch granule dyed by iodine. Black arrows indicate sites of microgranules, white arrows indicate holes in the starch granule. Scale bar: 33 µm (A); 25 µm (B); 2.0 µm (C). S, Starch granule; Ca, Cambium; Pc, Parenchyma cell. A image was from slender storage root, B and C images were from thick storage root.

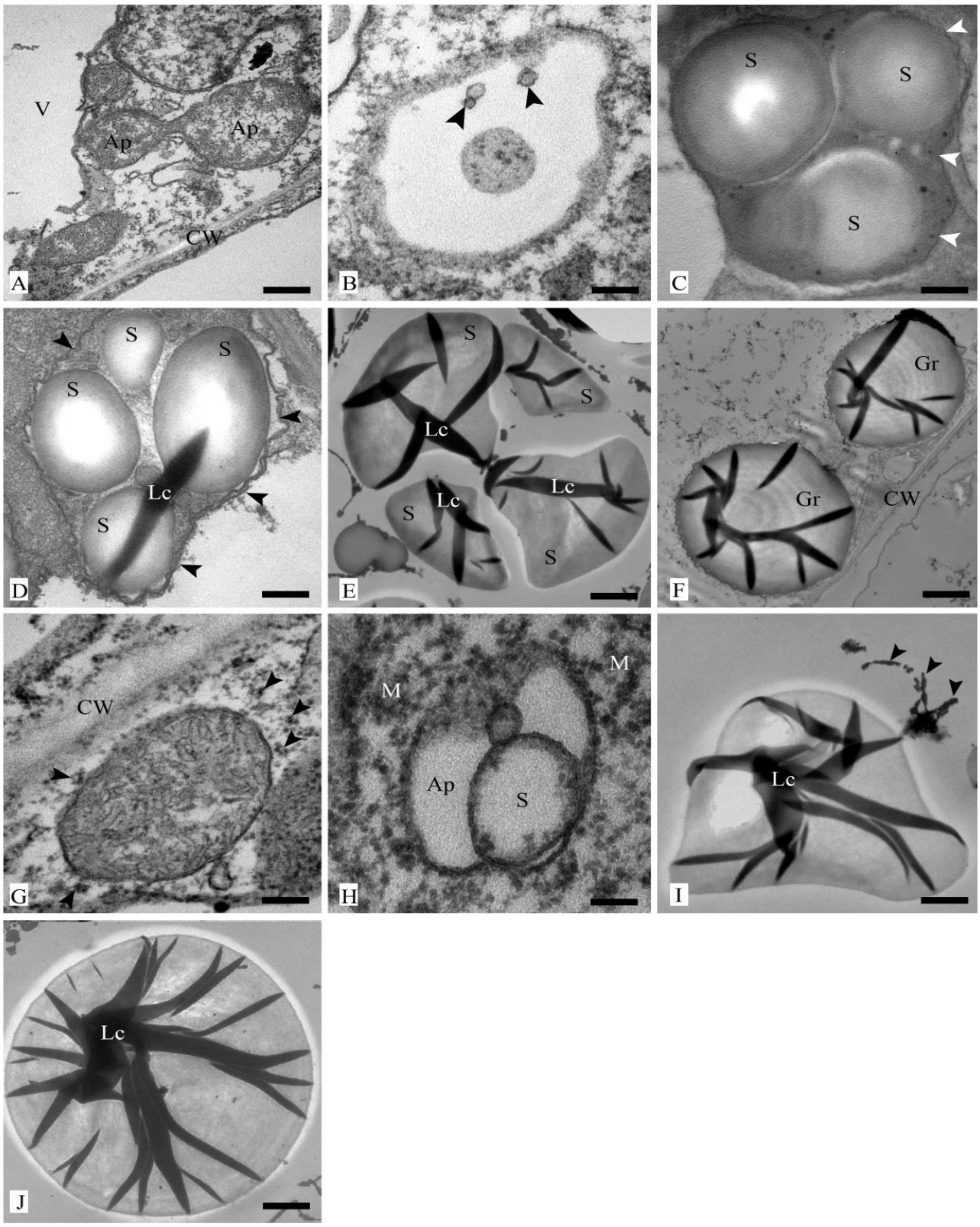

Fig. 2. TEM graphs showing growth of starch granule. A a division of amyloplast occurred in intermediate stages of growing amyloplasts. B. The amyloplast began synthesizing starch granules. C. The younger starch granules without crystals shared the same membrane, which is indicated by white arrow. D The membrane of amyloplast encapsulated the starch granule. E. As the starch granule enlarged, the outer membrane disappeared. F. Topographic images of the growth ring. G. Accumulation of numerous particles in stroma is only evident in some sections. H. In the early stage of formation of starch granule, several microgranules gathered around and entered the amyloplast. I. Microgranules in linear or cluster gradually accumulated; some of the double helices formed the lamella crystals in the starch granules. Black arrow indicates double helical array. J. Several lamellae of crystal became star-like, and size of the crystal increased. Scale bar: 380 nm(A); 200 nm(B); 300 nm(C); 455 nm(D); 1.54 μm(E);1.16 μm(F); 250 nm(G); 110 nm(H); 1.25 μm(I); 1.54 μm(J). CW, Cell wall; Ap, Amyloplast; S, Starch granule; M, Microgranule; V, Vacuole; LC, Lamella crystals; Gr, Growth ring. A, B, C, G and H images were from slender storage root, D, E, F, I and J images were from thick storage root.

Ultrastructure of starch granule in purple sweet potato: Development of the starch granule was observed in detail by TEM, which confirmed the results of light microscopy. Initially, the proplastid was surrounded by an electron-dense membrane (Fig. 2A). Proliferation in amyloplasts was investigated in purple sweet potato. The amyloplast split by constriction with resultant divisions into two halves (Fig. 2A). Amyloplast splitting by constriction resulted in increases in numbers of amyloplast. The amyloplast began synthesizing starch granules (Fig. 2B). Some observations indicated that at least one starch granule was included in the amyloplast. In fact, two and four starch granules, similar to the binary and tetrad structures, were more common in amyloplasts. The tetrad structure was more common in purple sweet potato, as compared with the binary structure. The size of the starch granule increased with the development of the tuber. Moreover, newly formed starch granules shared a common outer membrane (Fig. 2C, D), which disappeared after growth of the starch granule (Fig. 2E). Cells of the mature tuber contained several starch granules that were approximately 1–20 μm in size. Only few starch granules showed indication of growth rings in the present study (Fig. 2F).

Transmission electron microscopy (TEM) results showed that a large number of small spherical particles (20–100 nm) (Fig. 2G), which resemble 'microgranules', was formed in the stroma, using glucose yield from chloroplasts or chloroamyloplasts possessing a potentially functional photosynthetic apparatus. They accumulate around and enter the amyloplast, contributing to the formation of the starch granule (Fig. 2H). In the original formation of starch granule, there were no absolute crystals aggregated before the microgranules began to accumulate inside the starch granule (Fig. 2C). Thereafter, most of the microgranules lined up and moved towards the bulky starch granules in chains or clusters, polymerizing together. Some of the double helices formed the lamella crystals in the starch granules (Fig. 2I). Development of tuber resulted in the gradual accumulation of the microgranules in the starch granules, and crystals were formed, initially as a single lamella (Fig. 2D), followed by a cross arrangement of lamellae and increase in the number of lamellae (Fig. 2E). Several lamellae of crystals crossed to become star-like, and the size of crystal increased, which matched with the star-like crystals image by LM (Fig. 1B). The starch eventually accumulated at a high concentration, as shown in Fig. 2J. There was a hole in the centre of the starch granule (Fig. 2D).

The ultrastructure of starch granule from purple sweet potato root was studied by scanning electron microscopy (SEM). SEM showed that many microgranules adhered to the cell wall at the initial formation (Fig. 3A). Similarly, a large number of microgranules appeared near the amyloplast in the mature purple sweet potato tuber cells (Fig. 3B). Development of the tuber resulted in increased size and number of starch granules, and binary and tetrad structures were clearly observed, the latter being more common (Fig. 3C). The microgranule crossed the membrane of the starch granule (Fig. 3D) and fused with the blocklet crystals. The inner structure of starch granule was observed; there was a hole, crystal, and a small transmembrane microgranule in the centre of the starch granule (Fig. 3E), which are in agreement with the results of microstructure of starch granules. The spherical microgranules were attached to starch granule in the form of chains or double helical arrays (Fig. 3F). In addition, the surface of starch granule was covered by microgranules (20–100 nm), which are present in the images shown in Fig. 3A, F. They formed multiple concentric shells (or lamellae), resulting in an increased diameter of the starch granule and extension from the centre to the surface of granules (like onion's layers). The mature starch granules were about 1–20 μm in diameter, which was consistent with previous studies (Noda *et al*., 1992; Yong *et al*., 2018). These results verified the accumulation of starch observed by TEM.

Discussion

Previous studies have also shown that an electron-dense annulus existed in the constricted region of plastids (Chaly & Possingham, 1981; Hashimoto, 1986; Mingo-Castel *et al*., 1991; Yoder *et al*., 2001). The amyloplast divided from proplastid in the way of proliferation, and the proplastid elongated and subsequent constricted at random sites, with the resultant breaking of amyloplast dormancy (Sagisaka, 2008). Mingo-Castel *et al*., (1991) reported on amyloplast division, in which potato tubers were induced by kinetin and cultured *In vitro*. However, the results showed that amyloplast division only proceeded by binary fission of dumbbell-shaped amyloplasts, which was different from our findings of fission at random sites. The starch granules in purple sweetpotato were smaller than those found in the potato starch granules (100 μm), according to Descours *et al*., (2013) and Jenkins *et al*., (1993). Our results of growth rings in starch granules agreed with early electron microscopic observations of maize endosperm sections, in which less than 15% of the starch granules showed growth rings (Badenhuizen, 1959; Baker *et al*., 2001).

The whole stroma in purple sweet potato storage root was characterized by a number of particles in clusters that resemble features of stroma in oat coleoptiles (Hinchman, 1972). The development of the starch granule take place by coagulation at the periphery of material formed in the stroma, which was gradually transformed into the granule structure (Salema & Badenhuizen, 1967). Our results observed by TEM verified Salema's result too. Previous study described thick crystalline lamella as 'worm-like ripple structures' in corn starch granules (Yamaguchi *et al*., 1979). Baker *et al*., (2001) reported that the most obvious topographic features arranged in a radial fashion were likened to the spokes of a wheel. A previous study showed 5 nm natural starch granules, described as a dark birefringence cross, with the characteristic of crystalline matter (Tester *et al*., 2004). These 'spokes', 'worm-like

ripple structures', and dark birefringence are same as crystalline lamella. In addition, prior research reported various crystal formations. Yamaguchi *et al*., (1979) described some 'worm-like ripple structures', and crystalline lamella was associated with double helical amylopectin. The 'super-clusters' of starch were related to a dark birefringence cross, or the blocklet crystalline structure of starch (Tester *et al*., 2004). These 'spoke-like' crystals are due to compression and folding of starch (Baker *et al*., 2001). Our TEM images (Fig. 2I) revealed that the self-assembly lamella crystal was connected with microgranule folding in chain or clusters, in which amylopectin must be arranged in a double helical array. Some evidence indicated that the centre of the granule is depressed, where parts of the hilum were pulled out by the cutting knife (Baker *et al*., 2001). From our research, the pretreatment of TEM required the penetration of resin into the starch granule, including starch gaps. After filling the resin, the gap in the starch granules section was thinner than other parts of the starch granules. The same intensity of electron beam irradiation resulted in a visual hole. Further, the appearance of the hole depends on whether the location of the cross-section is central or non-central in the starch granule.

Early relevant work showed that the surface of starch granule consisted of small particles, called amylopectin blocklets (Sujka & Jamroz, 2009). According to Tester *et al*., (2004), these multiple concentric shells (or lamellae) were constructed by microgranules, such that the diameter from the centre to the surface of granules increased. The level of analytical sophistication helped in understanding the structure of starch. In our purple sweet potato research, 'microgranules' have been observed to form crystals and the exterior surface of the starch granule. The construction process of the starch granule and the formation of crystals and holes in the starch granules were similar to those of starch granules observed by TEM. Thus, we conclude that the microgranules arranged in a linear fashion, containing the double helix, is positively correlated with the formation of lamella crystals fraction, and irregular arrangement of microgranule form a close relationship with starch surface structure.

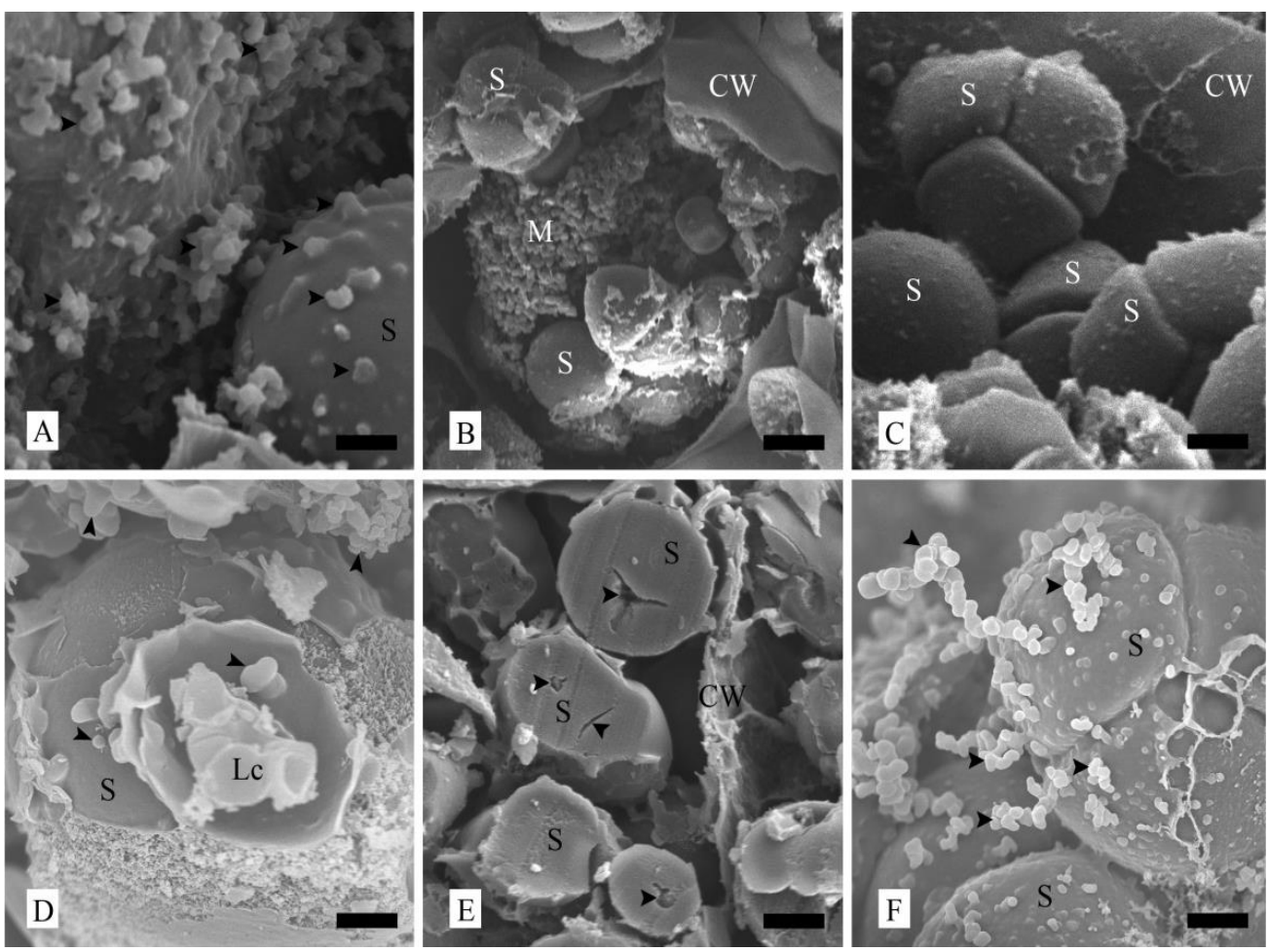

Fig. 3. SEM images showing the formation of starch granules in purple sweet potato. A. In the matrix, numerous microgranules stuck to the wall of cell, and black arrows indicate the sites of microgranules. B. Many microgranules in bulk form are attached to the starch granule. C. SEM image shows binary and tetrad structures of starch granules. D. Some microgranules passed through the membrane of the starch granule. Black arrows indicate the sites of the microgranules. E. Holes appeared in the centre of starch granules. Black arrows indicate sites of holes. F. SEM image of microgranule in linear or chain clusters accumulated with starch granules to constitute a part of starch granules. Black arrows indicate sites of microgranules in the organization of double helices. Scale bar: 3.0 μm(A); 13.5 μm(B); 6.9 μm(C); 1.67 μm(D); 56 μm(E); 4.35 μm(F). CW, Cell wall; S, Starch granule; M, Microgranule; LC, Lamella crystals. A image was from slender storage root, B, C, D, E and F images were from thick storage root.

Conclusions

This paper elucidated that the biological events during growth of starch granules, the structure of starch granules, and occurrence of the proliferation division amyloplasts. Micrographs of the purple sweet potato roots showed that starch from purple sweet potato appeared oval. However, there were star-shaped or needle crystalline bodies existing in the starch granules. After the development of the tuber and division activities, there was a subsequent increase in the number of amyloplasts, resulting in the formation of the starch granules. When the development reached the final stages, the size and number of the starch granules increased, and the binary and tetrad fission structures were easily visible by light microscope and electron microscopy. The probability of occurrence of tetrad fission was higher than that of binary fission. The newly forming granules shared the same layer as that of the outer membrane. Starch granules in purple sweet potato cell differed in their sizes (1-20 μm). TEM and SEM images of purple sweet potato tuber revealed that several microgranules (20–100 nm) moved toward and entered the bulky starch granules in chains or clusters and coalesced together. The initial observation showed that microgranules went through the membrane into the starch granules and ultimately formed cross lamellae crystals, with a resultant increase in sizes of the crystals because of numerous microgranules aggregating in chain or clusters. Various lines of evidence indicated that the surface of starch granule was also constructed by microgranules, leading to the formation of the starch growth shell. When the shell extended from the centre to the surface of granules, the diameters of the starch granules increased.

Acknowledgements

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References

Badenhuizen, N.P. 1959. Chemistry and Biology of the Starch Granule: Springer Vienna.

Baker, A.A., M.J. Miles and W. Helbert. 2001. Internal structure of the starch granule revealed by AFM. *Carbohyd. Res.*, 330(2): 249-256.

Ball, S., H.P. Guan, M. James, A. Myers, P. Keeling, G. Mouille and J. Preiss. 1996. From glycogen to amylopectin: a model for the biogenesis of the plant starch granule. *Cell*, 86(3): 349.

Ball, S.G. 1995. Recent views on the biosynthesis of the plant starch granule. *Trends in Glycosci. & Glycotech*., 7(37): 405-415.

Cameron, R. and A. Donald. 1992. Small-angle X-ray scattering study of the annealing and gelatinization of starch. *Polymer*, 33(12): 2628-2635.

Chaly, N. and J.V. Possingham. 1981. Structure of constricted proplastids in meristematic plant tissues. *Biol. Cell*, 41(3): 203.

Denyer, K.A.Y., P. Johnson, S. Zeeman and A.M. Smith. 2001. The control of amylose synthesis. *J. Plant Physiol.*, 158(4): 479-487.

Descours, E., A. Hambleton, M. Kurek, F. Debeaufort, A. Voilley and A.M. Seuvre. 2013. Aroma behaviour during steam cooking within a potato starch-based model matrix. *Carbohyd. Polym*., 95(1): 560-568.

French, D. 1984. Organization of starch granules. *Starch: Chem. & Technol.*, 2: 183-247.

Gibbons, I.R. and A.V. Grimstone. 1960. On flagellar structure in certain flagellates. *J. Biophys. Biochem*. *Cytol.,* 7(4): 697.

Hashimoto, H. 1986. Double ring structure around the constricting neck of dividing plastids of Avena sativa. *Protoplasma*, 135(2): 166-172.

Hinchman, R.R. 1972. Ultrastructural morphology and ontogeny of oat coleoptile plastids. *Amer. J. Bot.,* 59(8): 805-817.

Jenkins, P.J., R.E. Cameron and A.M. Donald. 1993. A universal feature in the structure of starch granules from different botanical sources. *Starch/Starke,* 45(12): 417-420.

Mingo-Castel A.M., A.M., Pelacho and M.R. de Felipe 1991. Amyloplast division in kinetin induced potato tubers. *Plant Sci*., 73(2): 211-217.

Montgomery E.M. and F. Senti. 1958. Separation of amylose from amylopectin of starch by an extraction-sedimentation procedure. *J. Polym. Sci*., 28(116): 1-9.

Nikolakaki, A. and N.S. Christodoulakis. 2007. Secretory structures and cytochemical investigation of the leaf of Phlomis fruticosa, a seasonally dimorphic subshrub. Secreting activity of the leaf-originating calluses. *Flora,* 202(6): 429-436.

Noda T., Y. Takahata and T. Nagata. 1992. Developmental changes in properties of sweet potato starch. *Starch/Stärke*. 44: 405-409.

Sagisaka, S. 2008. The proliferation of amyloplasts in meristematic cells of developing stolons of potato and apple callus: progenitors of proplastids. *J. Plant Physiol*., 165(16): 1678-1690.

Salema, R. and N.P. Badenhuizen. 1967. The production of reserve starch granules in the amyloplasts of *Pellionia daveauana* N. E. Br. *J. Ultrastruc. Res*., 20(5): 383-399.

Sujka, M. and J. Jamroz. 2009. α-Amylolysis of native potato and corn starches–SEM, AFM, nitrogen and iodine sorption investigations. *LWT-Food Sci. Technol*., 42(7): 1219-1224.

Tang, H., T. Mitsunaga and Y. Kawamura. 2006. Molecular arrangement in blocklets and starch granule architecture. *Carbohyd. Polym*., 63(4): 555-560.

Tester, R.F., J. Karkalas and X. Qi. 2004. Starch-composition, fine structure and architecture. *J. Cereal Sci*., 39(2): 151-165.

Tumuhimbise, G.A., A. Namutebi and J.H. Muyonga. 2009. Microstructure and in vitro beta carotene bioaccessibility of heat processed orange fleshed sweetpotato. *Plant Foods Human Nutr*., 64(4): 312-318.

Wang, S., J. Yu, F. Jin and J. Yu. 2008. The new insight on ultrastructure of C-type starch granules revealed by acid hydrolysis. *Int. J. Biol. Macromol*., 43(2): 216-220.

Wilson, L.A. and S.B. Lowe 1973. The Anatomy of the Root System in West Indian Sweet Potato (*Ipomoea batatas* (L.) Lam.) *Cultivars. Ann. Bot*., 37: 633-43.

Yamaguchi, M., K. Kainuma and D. French. 1979. Electron microscopic observations of waxy maize starch. *J. Ultrastruc. Res.*, 69(2): 249-261.

Yoder, T.L., H. Zheng, P. Todd and L.A. Staehelin. 2001. Amyloplast sedimentation dynamics in maize columella cells support a new model for the gravity-sensing apparatus of roots. *Plant Physiol.,* 125(2): 1045-1060.

Yong, H.M., X.C. Wang, J. Sun, Y. Fang, J. Liu and C.H. Jin. 2018. Comparison of the structural characterization and physicochemical properties of starches from seven purple sweet potato varieties cultivated in China. *Int. J. Biol. Macromol*., 120: 1632-1638.

Zhang, B., N. Selway, K.J. Shelat, S. Dhital, J.R. Stokes and M.J. Gidley. 2017. Tribology of swollen starch granule suspensions from maize and potato. *Carbohyd. Polym*., 155: 128-135.

Zhang, H., L. Wang, S. Deroles, R. Bennett and K. Davies. 2006. New insight into the structures and formation of anthocyanic vacuolar inclusions in flower petals. *BMC Plant Biol*., 6(1): 29-43.

Zhang, L., L.L. Zhao, X.F. Bian, K. Guo, L. Zhou and C.X. Wei. 2018. Characterization and comparative study of starches from seven purple sweet potatoes. *Food Hydrocolloids*, 80: 168-176.

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