Chapter 13
Morphological Variations of Starch Grains

Ryo Matsushima

Abstract Starch is synthesized in plant storage organs and forms transparent grains inside cells, which are referred to as starch granules or starch grains (SGs). SGs exhibit different morphologies and sizes depending on the species and are prominent in Poaceae endosperm. Comprehensive observations indicate that SG morphologies can be classified into four types: compound grains, bimodal simple grains, uniform simple grains, and a mixed configuration containing compound and simple grains in the same cells. Phylogenetic evaluation of SG morphological diversity indicates that the compound grain type is the ancestral SG morphology in Poaceae, and the bimodal simple grain type is only observed in specific phylogenetic groups that include barley and wheat. Starch morphology and size are important characteristics for industrial applications. However, the molecular mechanisms that determine SG morphology and size are not completely understood. This review summarizes starch grain morphological characteristics and phylogenetic information about SG morphological diversity. It also discusses methods for cytological observation of SGs and recently identified genes that control SG size.

Keywords Cereal • Diversity • Endosperm • Mutant • Starch grain • Voronoi diagram

13.1 What Is a Starch Grain?

Starch is a biologically important glucose polymer that is synthesized by photosynthetic organisms such as plants and algae (Buléon et al. 1998; Hancock and Tarbet 2000; Nakamura 2002). Starch contains amylose and amylopectin. Amylose is primarily a linear glucose chain, whereas amylopectin is a densely branched glucose chain. Amylopectin is the major component of normal starch, accounting for 65–85 % of total starch weight. Starch is water-insoluble and osmotically inactive, making it a suitable long-term storage form of carbohydrate for seeds and tubers of

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many plant species. The higher plant amyloplast, a terminally differentiated plastid, is the site of starch synthesis and storage in organs such as endosperm, potato tubers, and pollen grains from Poaceae (James et al. 2003; Sakamoto et al. 2008). Starch forms transparent grains in amyloplasts, which are referred to as starch granules or starch grains (SGs). High levels of starch accumulate in storage organs, where most of the amyloplast interior is occupied by SGs. SGs are easily visualized using iodine solution and can be clearly observed under a normal light microscope (Matsushima et al. 2010).

13.2 Morphological Variations of Starch Grains

Although starch has a simple glucose polymer composition, SGs exhibit differing morphologies depending on the species (Harz 1880; Tateoka 1954, 1955, 1962; Czaja 1978; Jane et al. 1994; Shapter et al. 2008; Matsushima et al. 2010). SG morphology is classified as either compound or simple (Tateoka 1962; Grass Phylogeny Working Group 2001). For both classes, only one SG is formed in one amyloplast. The difference is that the compound SGs are assembled from several dozen small starch granules, whereas the simple SG is a single particle of one starch granule (Fig. 13.1a). The SG size for simple SGs is equal to that of the starch granules. SGs in Poaceae endosperm have been extensively observed and their morphologies have been comprehensively described (Tateoka 1962; Grass Phylogeny Working Group II 2012). Rice (*Oryza sativa*) endosperm develops compound SGs that are normally 10–20 μm in diameter (Fig. 13.1b, e, h). Each starch granule included in compound SG is a sharp-edged polyhedron with a typical diameter of 3–8 μm. Starch granules are assembled as a compound SG, but they are not fused and are easily separated by conventional purification procedures. Simple SGs are observed in several important crops such as maize (*Zea mays*), sorghum (*Sorghum bicolor*), barley (*Hordeum vulgare*), and wheat (*Triticum aestivum*). Simple SGs are further classified into two subtypes referred to as bimodal and uniform. The bimodal type contains small and large simple SGs that coexist in the same cells. The uniform type contains similar-sized hexagonal, pentagonal, or round simple SGs. Barley and wheat synthesize bimodal simple SGs, whereas maize and sorghum synthesize uniform simple SGs (Fig. 13.1c, f, i, d, g, j). A number of species such as *Miscanthus*, *Perotis*, *Gymnopogon*, and *Thuarea* display a mixed configuration of compound and simple SGs inside the same cell (Tateoka 1962). Phylogenetic considerations about SG morphological diversity in Poaceae will be discussed later. Compound and simple SGs are also observed in tubers. Potato (*Solanum tuberosum*) and Chinese yam (*Dioscorea batatas*) synthesize simple SGs, whereas compound SGs are observed in sweet potato (*Ipomoea batatas*) and eddoe (*Colocasia esculenta*) (Fig. 13.2).

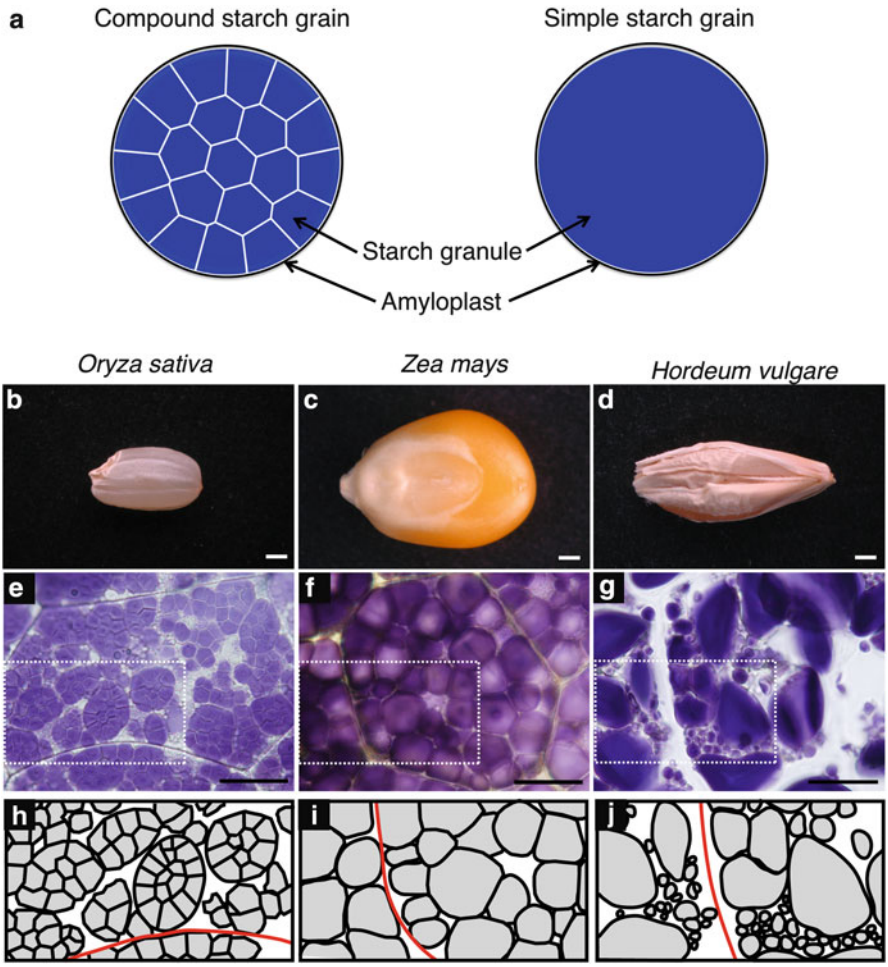


Fig. 13.1 Compound and simple starch grain characteristics. (**a**) Cartoon comparing compound and simple starch grains (SGs). SGs are synthesized and stored in amyloplasts. Compound SG is an assembly of several dozen small starch granules, whereas simple SG is a single particle of one starch granule. (**b**–**d**) Rice (*Oryza sativa*), maize (*Zea mays*), and barley (*Hordeum vulgare*) seeds. Bars = 1 mm. (**e**–**g**) Iodine-stained SGs in endosperm thin sections. Bars = 20 μm. Boxes indicated by white dotted lines are illustrated in **h**–**j**. (**h**–**j**) Illustration of typical SGs of each species. Rice, compound SGs; maize, uniform simple SGs with similar spherical sizes; barley, bimodal simple SGs containing small and large SGs coexisting in the same cell. Red lines indicate cell walls (Modified from Figure 2 in Matsushima et al. 2010)

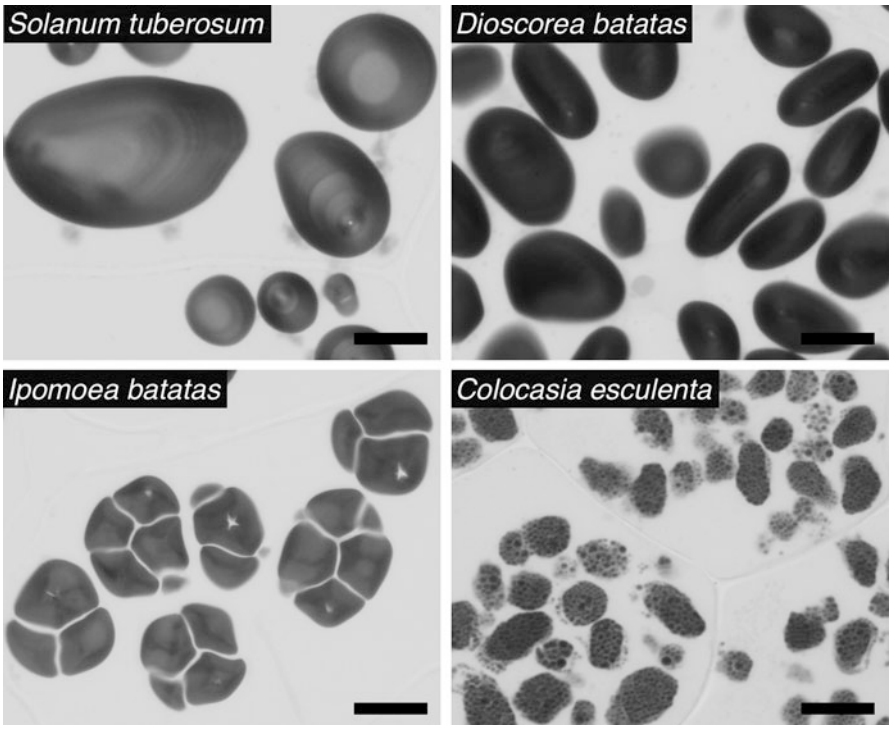


Fig. 13.2 Starch grains in tubers. Potato (*Solanum tuberosum*) and Chinese yam (*Dioscorea batatas*) develop simple SGs, whereas sweet potato (*Ipomoea batatas*) and eddoe (*Colocasia esculenta*) develop compound SGs. Bars = 20 μm

13.3 Phylogenetic Characteristics of Starch Grain Morphologies

Although SG morphological diversity has been observed and described, a detailed examination of the phylogenetic origin is lacking. Recent work extensively reevaluated previous SG observations in Poaceae endosperm for alignment on the newest Poaceae phylogenetic tree (Matsushima et al. 2013) (Fig. 13.3). This work showed that Poaceae early diverging genera, such as *Pharus*, *Anomochloa*, and *Streptochaeta*, synthesize compound SGs, indicating that compound SG is plesiomorphic in Poaceae. Genera with uniform simple SGs are scattered across phylogenetic branches, and this type is most frequent within the tribes Paniceae, Andropogoneae, and Paspaleae. By contrast, bimodal simple SGs were restricted to only five genera, including *Brachypodium*, *Triticum*, *Hordeum*, *Elymus*, and *Bromus*. This indicates that bimodal simple SGs are phylogenetically specific for Poaceae. Currently, there is no information about genetic mechanisms that change SG morphological types. This information will reveal the evolution of SG morphological specification and explain the current phylogenetic profile.

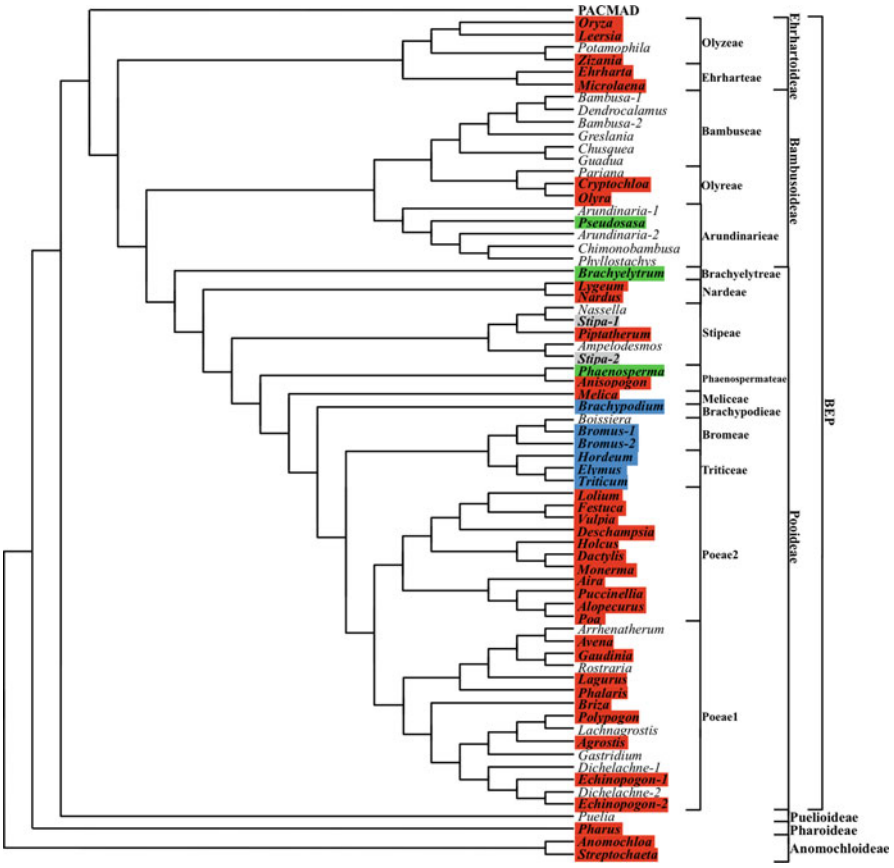
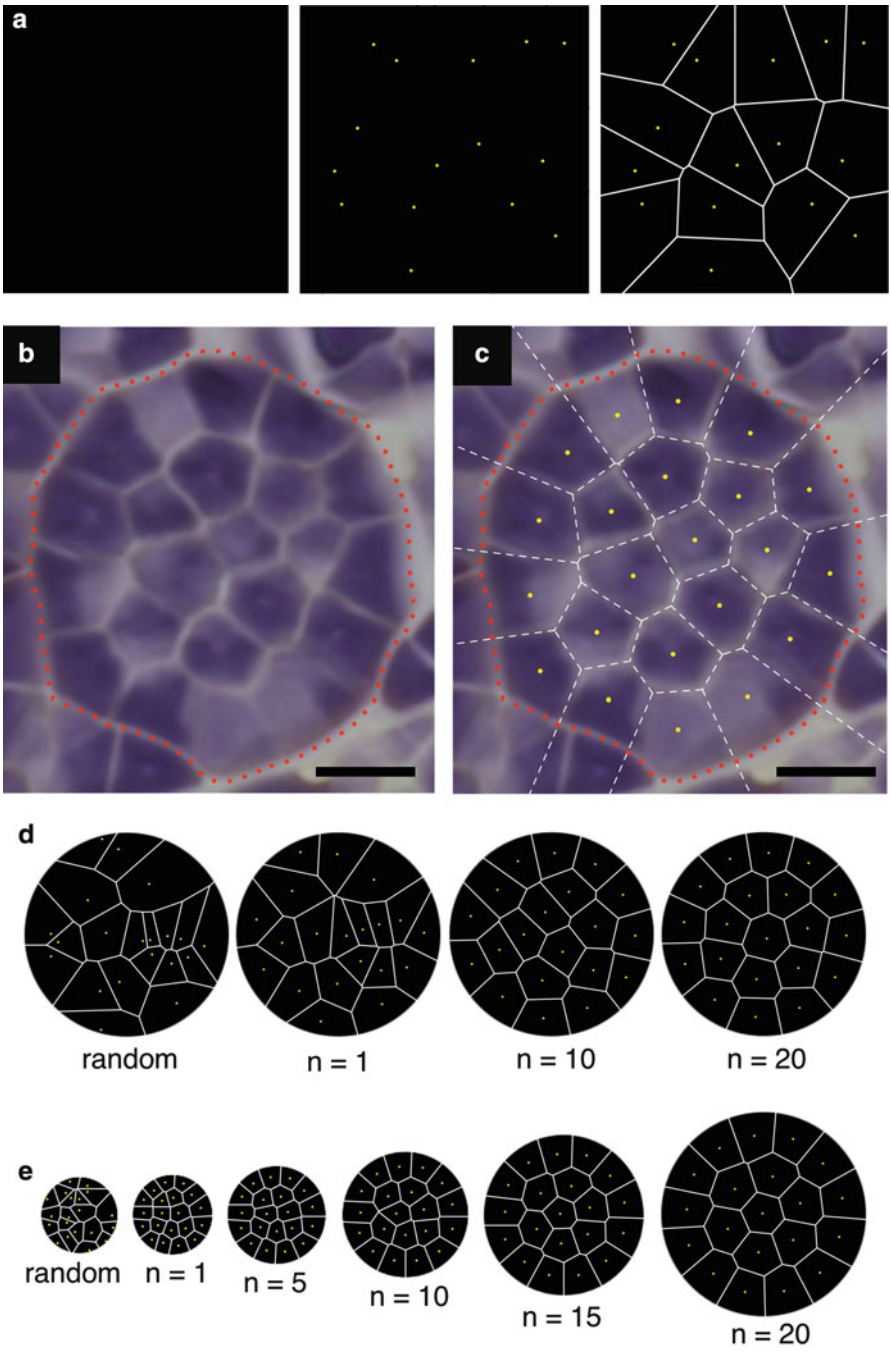


Fig. 13.3 Phylogenetic tree of starch grain morphologies in Poaceae. This tree is based on the Bayesian consensus phylogenetic tree constructed by Grass Phylogeny Working Group II (2012). Genera are color coded for SG morphological type: red, compound SG; blue, bimodal simple SG; green, uniform simple SG; and gray, genera with discrepancies between observations and genera with several SG types reported. For details of PACMAD clade, see supplemental data of Matsushima et al. (2013). BEP, subfamilies Bambusoideae, Ehrhartoideae, and Pooideae; PACMAD, subfamilies Panicoideae, Arundinoideae, Chloridoideae, Micrairoideae, Aristidoideae, and Danthonioideae (Modified from Figure 1 in Matsushima et al. 2013)

13.4 Voronoi Diagram of Compound Starch Grain Interior

Compound SGs are well-ordered structures, in which assembled starch granules are sharp-edged polyhedrons with similar diameters. In thin sections, the starch granule pattern in compound SGs is well fitted to the Voronoi diagram (Fig. 13.4). Voronoi diagrams are a mathematical representation of tessellations developed by Peter Gustav Lejeune Dirichlet (1805–1859) and Georges Voronoï (1868–1908) (Okabe et al. 2000). Given a set of generator points, the Voronoi tessellation


a
b
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n = 1
n = 10
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e
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n = 1
n = 5
n = 10
n = 15
n = 20

divides space into regions called Voronoi regions (Fig. 13.4a). A Voronoi region should consist of all points that are closer to the generator point in the region than to any other generator points (Okabe et al. 2000; Poupon 2004). To satisfy this condition in two-dimensional planes, the Voronoi region is built by defining the perpendicular bisector between the generator points and selecting the smallest polyhedron formed by these lines. Given the appropriate sets of generator points in thin sections, the area of each starch granule in compound SGs corresponds to Voronoi regions (Fig. 13.4b, c). Agreement between Voronoi diagrams and starch granule shapes indicates two properties about compound SG development. First, during SG synthesis in developing seeds, the enlargement rate of each starch granule is almost equal. Second, the formation of all starch granules starts simultaneously. Otherwise, smaller starch granules than those expected by Voronoi regions would be generated. When the starch granules are observed under polarized light, a characteristic dark cross can be visualized (Pérez and Bertoft 2010). The center of the cross is called hilum, the point around which layers of carbohydrate are deposited (Buléon et al. 1998; D'Hulst and Merida 2010). The hilum is suggested to prime starch synthesis; in this respect, the starch granule hilum may correspond to the generator points in Voronoi regions.

As starch granules enlarge in developing endosperm, they affect the growth of neighboring granules and impose limits on intergranule spacing. This optimizes starch granule packing in amyloplasts and enables formation of densely packed compound SGs. A centroidal Voronoi tessellation has generator points as the centroids (centers of mass) of the corresponding Voronoi regions (Du et al. 1999). When the circle is divided by Voronoi tessellations with randomly located generator points, irregularly sized Voronoi regions are constructed (Fig. 13.4d). However, when the centroids of each Voronoi region are used as the updated generator points, a new Voronoi tessellation can be obtained. The iteration between constructing Voronoi tessellations and calculating centroids can achieve the centroidal Voronoi

Fig. 13.4 Voronoi diagrams of compound starch grains. (**a**) Voronoi tessellation of square (indicated by *black*). Given 15 generator points (*yellow points*), Voronoi tessellation divides the square into 15 Voronoi regions (polygons separated by *white lines*). A Voronoi region consists of all points that are closer to the generator point in the region than to any other generator point. (**b**, **c**) Compound SG (indicated by *red dotted line*) in wild-type rice endosperm (**b**) overlaid with a Voronoi diagram (*white dotted lines*) based on the *yellow* generator points (**c**), which were manually selected. Voronoi diagram was generated by R program using package EBImage, png, and deldir (Team 2013; Pau et al. 2014; Urbanek 2013; Turner 2014). Bars = 5 μm. (**d**) Lloyd method to obtain centroidal Voronoi tessellation for a circle. Voronoi tessellation divides the circle into 20 regions based on 20 generator points that are randomly located in the circle. By iterations ($n = 1$, 10, and 20) of calculating centroids and constructing Voronoi, tessellations converge into a centroidal Voronoi tessellation with well-shaped uniformly sized regions. After 50 iterations, the Voronoi tessellation resembles compound SGs. (**e**) Convergence into the centroidal Voronoi tessellation for an expanding circle. The iteration ($n = 1$, 5, 10, 15, and 20) is 5 % expansion of the circle, calculating centroids, constructing Voronoi tessellations. *Yellow points* indicate generator points of Voronoi tessellation

tessellation with well-shaped, uniformly sized regions: this is the Lloyd method (Du et al. 1999). The centroidal Voronoi tessellation for a circle resembles compound SGs. In developing endosperm, the starch granule enlargement occurs together with amyloplast size expansion. Therefore, centroid position in each starch granule should change depending on the amyloplast size expansion during endosperm development (Fig. 13.4e).

13.5 Observation Methods for Starch Grains

Starch turns violet when stained by iodine solution. Therefore, SGs can be easily observed under a light microscope. Thin sections can be obtained by hand sectioning samples with high water content such as potato tubers, which preserves intact subcellular structures. Staining the sections with iodine solution will label intact SGs inside cells. Thin sections from dry and hard materials such as cereal grains are difficult to prepare by hand sectioning. Cereal grains can be broken into fragments using pliers, and these can be stained with iodine solution to observe SGs. However, crushing by pliers can damage the compound SGs and disperse starch granules.

A rapid and easy method to prepare thin sections for SG analysis from cereal grains was developed recently (Matsushima et al. 2010). Using this method, compound SG morphologies can be observed in an intact state. This method is illustrated in Fig. 13.5. The tip of a 200 μL pipette tip is cut off using scissors (Fig. 13.5a). The cut end is softened by mechanical pinching. A seed can fit into the pinched end for fixation. For rice, barley, and wheat, 200 μL tips are suitable for fixation of seeds. For maize, 1 mL pipette tips are suitable. The seed-embedded tip can be fixed on a block trimmer used for ultramicrotome resin block trimming (Fig. 13.5b) and manipulated under a stereo microscope (Fig. 13.5c) for thin sectioning under magnified observation. The block trimmer can be held by the third and fourth fingers of the nondominant hand, whereas the index finger and thumb of the dominant hand hold a razor blade attached to the seed (Fig. 13.5d). During sectioning, the blade is kept horizontal to the seed. The blade should be supported by the index finger of the nondominant hand to adjust sectioning pressure. The razor cut generates a smooth surface near the top of the seed, which is exposed approximately 1 mm out of the pipette tip (Fig. 13.5e, f). Thin sectioning can be performed with the same hand positions, but holding the blade at an angle of approximately 30° (Fig. 13.5g). The seeds are easily cut using a razor blade, and thin sections can be shaved off the cut end (Fig. 13.5h, i). The razor blade must be sharp for this procedure. The thin section can be picked up by forceps and placed onto a glass slide for iodine staining. This rapid method is easy and suitable for observation of large numbers of samples, such as more than 200 seeds per day. This renders the method an excellent choice for genetic screening of mutants. The embryo remains intact after sectioning if the embryo-containing end is embedded into the tip. Therefore, the sectioned seed can still germinate. There are two disadvantages to the rapid method. It cannot create a large thin section, so a wide-field view at

low magnification cannot be obtained. This method cannot be used to create thin sections from seeds with a floury quality such as floury mutant seeds.

To address the disadvantages of the rapid method, Technovit 7100 resin can be used (Matsushima et al. 2010; Matsushima et al. 2014). Technovit 7100 resin is a low-temperature polymerization resin composed primarily of glycol methacrylate that is available from Heraeus-Kulzer (Germany). In the resin-embedded method, approximately 1 mm cubic blocks of endosperm are subjected to chemical fixation. Samples are subsequently dehydrated through a graded ethanol series and then embedded in Technovit 7100 resin (http://www.bio-protocol.org/e1239). The embedded samples are cut with an ultramicrotome and diamond knife and dried on cover slips. The thin sections are approximately 1 μm thick and can be stained with iodine solution. The resin-embedded method takes approximately 1 week to perform. It is not suitable for large numbers of samples but can produce larger sections than the rapid method and can produce thin sections from floury seeds. The resin-embedded samples are competent for long-term preservation and provide higher-resolution images compared with those of the rapid method.

13.6 Mutants Isolated by Direct Observations of Starch Grains

Molecular mechanisms that determine organelle shape have been studied using genetic approaches and live-cell imaging in *Arabidopsis*. Organelles such as vacuoles, endoplasmic reticulum, Golgi apparatus, mitochondria, and peroxisomes can be visualized by expressing organelle-targeted green fluorescent protein (GFP) in transgenic plants (Mano et al. 2009). This technique enables direct observation of organelles in live plant cells. Live-cell imaging is suitable for genetic screening of mutants showing abnormal organelle morphologies. Molecular characterization of isolated mutants has identified molecular mechanisms involved in determining organelle morphology (Mano et al. 2004; Matsushima et al. 2004; Tamura et al. 2005; Arimura et al. 2008; Nakano et al. 2012).

SGs in mature cereal seeds have not been clearly visualized using live-cell imaging techniques. The rapid method for SG observations in thin sections can be used for genetic screening of mutants. The rapid method utilizes only half of the endosperm and preserves the embryo; therefore, assayed seeds maintain germination competence. The rapid method was successfully employed to isolate five rice mutants with abnormal SG morphology (Fig. 13.6). These mutants were designated *ssg* for *substandard starch grain*. The *ssg1*, *ssg2*, and *ssg3* mutants display higher levels of smaller SGs (<10 μm in diameter) in addition to normal SGs (10–20 μm in diameter). The *ssg4* mutant contains enlarged compound SGs (>30 μm in diameter). The *ssg5* mutant contains SGs that lack characteristic compound structures.

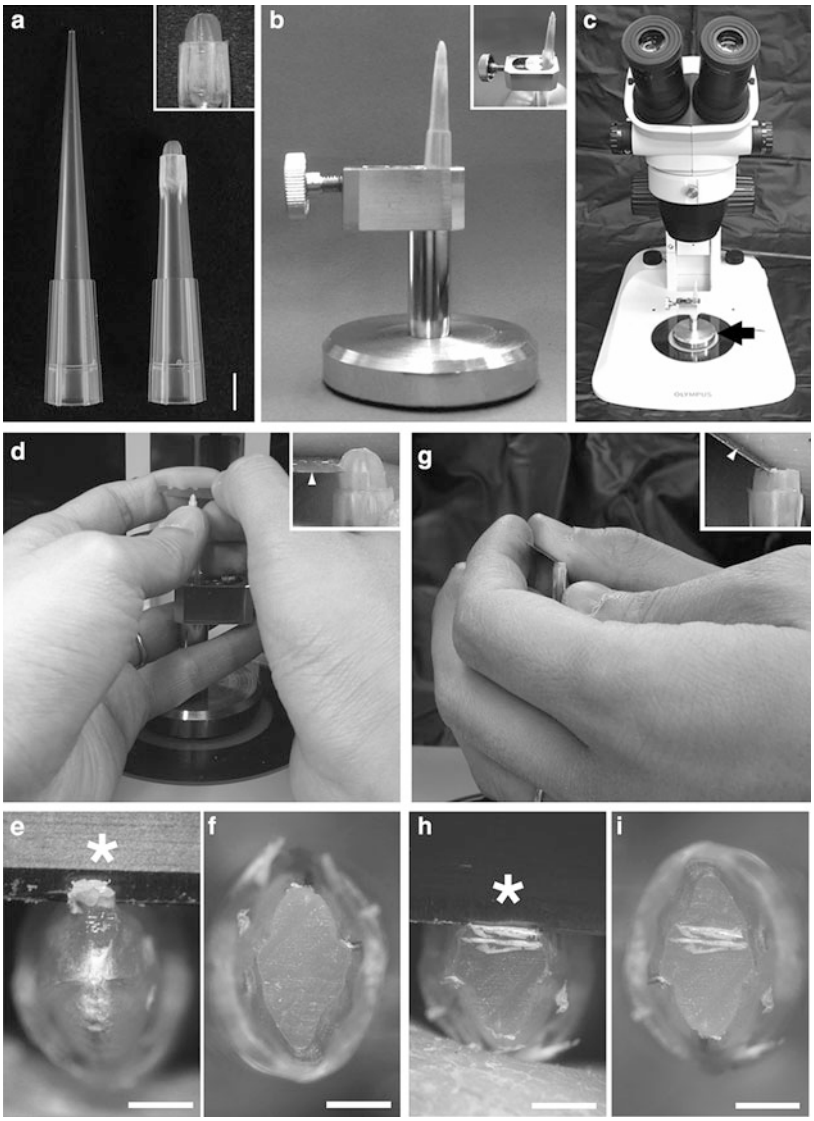

Fig. 13.5 Rapid preparation of rice endosperm thin sections for starch grain observation. (**a**) A rice seed was inserted into a truncated, disposable pipette tip (*right*) (inset, magnified image of the seed). Bar = 5 mm. (**b**) The seed-containing tip was fixed on an ultramicrotome block trimmer (inset, overhead view). (**c**) The block trimmer (indicated by an *arrow*) can be manipulated under a stereo microscope. (**d**) Positions of hands, fingers, razor blade, and the block trimmer equipped with the seed-containing tip for trimming (inset, magnified side view of the captured seed and razor blade, indicated by *arrowhead*). (**e**) Seed images under stereo microscope before trimming (*asterisk* indicates razor blade). Bar = 1 mm. (**f**) Smooth surface after trimming. Bar = 1 mm. (**g**) Side view of hand positions for thin sectioning. Razor blade angle is approximately 30° (inset, *arrowhead*). (**h**, **i**) A thin section can be shaved off using the razor blade (indicated by an *asterisk* in **h**). Bars = 1 mm (Modified from Figure 1 in Matsushima et al. 2010)

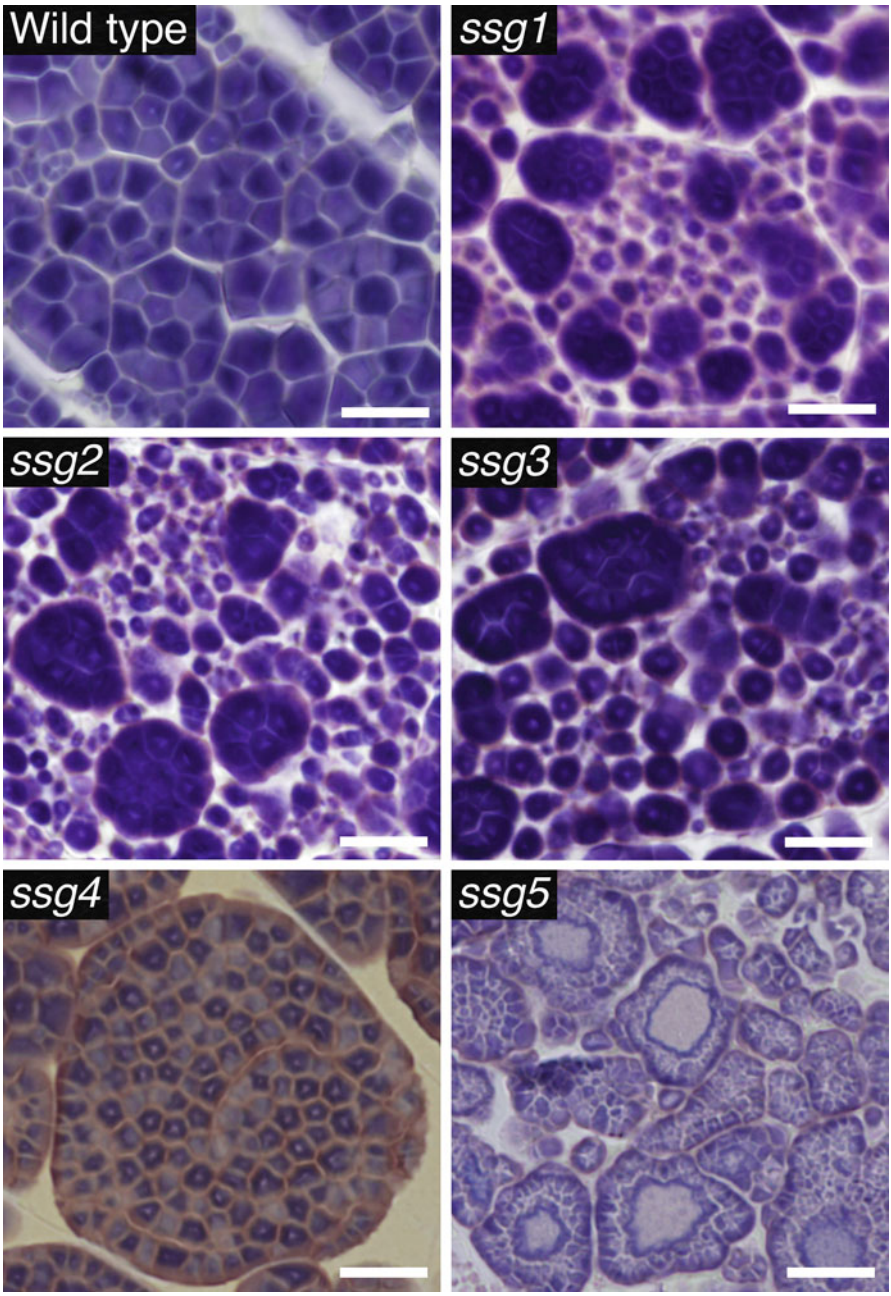

Fig. 13.6 Rice *substandard starch grain* (*ssg*) mutants with abnormal starch grain morphologies. The *ssg1*, *ssg2*, and *ssg3* mutants contain higher levels of smaller SGs. Enlarged compound SGs are detected in *ssg4*. SGs without internal compound structures are observed in *ssg5*. Bars = 10 μm (Modified from Matsushima et al. 2010 and Matsushima et al. 2014)

13.7 Size Variations of Starch Grains

The size of SGs and starch granules is one of the most important starch characteristics for industrial applications (Lindeboom et al. 2004). Small starch granules can replace fat in food applications, because aqueous dispersions of small starch granules show fat mimetic properties (Malinski et al. 2003). Larger starch granules are desirable in maize and cassava because they improve the final yield after wet-milling purification (Gutiérrez et al. 2002). Therefore, engineered size control of SGs and starch granules is a molecular tool that can be used for starch breeding in future biotechnology programs.

SG size in cereal endosperm is diverse and species-specific. *Bromus* species contain intrageneric size variations of SGs, in which even phylogenetic neighbors develop distinctly sized SGs (Matsushima et al. 2013). This indicates that a small number of genes regulate the interspecific SG size variation. As the bimodal simple SGs, barley and wheat SGs contain two discrete size classes of approximately 15–25 μm and <10 μm that coexist in the same cells (Fig. 13.1). The percentage of smaller and larger SGs of bimodal simple SGs varies depending on species. In *Hordeum pusillum*, small SGs are highly abundant compared with other species. In contrast, small SGs are rare in *Elymus caninus* (Matsushima et al. 2013). Genetic factors controlling the numbers of small and large SGs of bimodal SGs have not been identified so far. However, small SGs are known to lack in a few wild wheat species, and a major QTL controlling the small SGs number has been detected (Howard et al. 2011).

13.8 Genetic Factors to Control Starch Grain Size

Four kinds of enzymes have major roles in starch biosynthesis, including ADP-glucose pyrophosphorylase (AGPase), starch synthase (SS), branching enzyme (BE), and debranching enzyme (DBE) (see Chaps. 5 and 6 in this book). These enzymes have multiple isoforms with different substrate specificities and distinct expression patterns. A number of mutants defective in starch biosynthetic enzymes have been isolated in several plant species (Dvonch et al. 1951; Walker and Merritt 1969; Jarvi and Eslick 1975; Satoh and Omura 1981; Yano et al. 1984; Satoh et al. 2003a, b, 2008; Kang et al. 2005; Fujita et al. 2007, 2009). Some of these mutants exhibit distinct sizes of SGs compared to those of the wild-type plants. Mutations in *BEIIb* mutants are designated *amylose extender* (*ae*) (Yano et al. 1985). Rice and maize *ae* endosperm contains smaller SGs (Yano et al. 1985; Li et al. 2007). *ssg1*, *ssg2*, and *ssg3* mutants also contain *BEIIb* mutations (Matsushima et al. 2010).

Mutations in *Arabidopsis SSIV* cause synthesis of one large starch granule per chloroplast in leaves (Roldán et al. 2007). SSIV may be involved in starch granule initiation and priming of amylopectin synthesis (Szydlowski et al. 2009; D'Hulst and Merida 2010). However, the role of SSIV in cereal endosperm is unknown.

Granule-bound starch synthase I (GBSSI) mutants are *waxy* mutants that produce no amylose (Fasahat et al. 2014). In barley and rice, SG size in *waxy* mutants

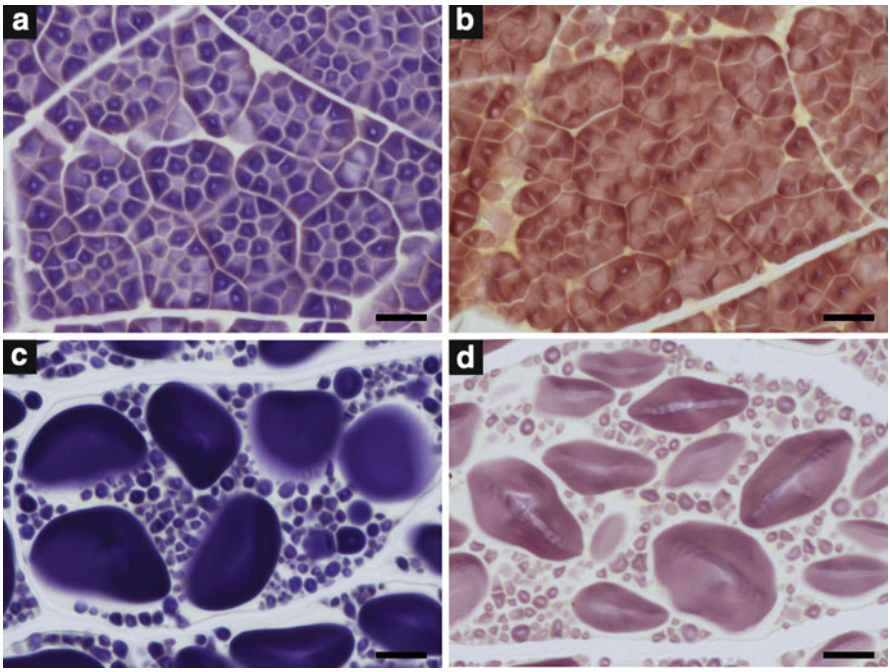

Fig. 13.7 Starch grain morphologies in *waxy* mutants of rice and barley. SGs from non-waxy seeds were stained violet, whereas SGs from *waxy* mutants were stained red. (**a**) Koshihikari variety of rice (non-waxy). (**b**) Odorokimochi variety of rice (waxy). (**c**) Akashinriki variety of barley (non-waxy). (**d**) Tokushima Mochimugi 1 variety of barley (waxy). The barley accessions were provided by the National Bio-Resource Project – barley of the MEXT, Japan. I especially thank Dr. Kazuhiro Sato (Institute of Plant Science and Resources, Okayama University) for providing the seeds. Bars = 10 μm

is comparable to that of wild-type (Fig. 13.7). Therefore, SG size is not directly determined by the amount of amylose.

Most of the amyloplast interior is occupied by SG in storage organs, where the SG is approximately the same size as the amyloplast. Amyloplasts and chloroplasts both develop from proplastids (Sakamoto et al. 2008). The size of chloroplast is controlled by the chloroplast binary fission division machinery, especially by the ring structures that form at the division sites (Miyagishima 2011; TerBush et al. 2013). Proteins involved in the ring structures have been isolated, including FtsZ, MinD, MinE, and ARC5 (DRP5B). *Arabidopsis* mutants that are defective in these proteins have defects in chloroplast division and contain enlarged chloroplasts. Overexpression of FtsZ1 in potato results in partial inhibition of plastid division in tubers (de Pater et al. 2006). The tubers generate fewer but larger amyloplasts and hence larger simple SGs. The diameters of SGs are approximately 1.2–1.7-fold larger in these transgenic potato plants than in wild-type plant. In rice, the *arc5* mutant is the only mutant reported to be defective in chloroplast division (Yun and

Kawagoe 2009). The *arc5* endosperm produces pleomorphic amyloplasts, but not enlarged ones. So, the size of SGs is little affected in rice *arc5* endosperm.

Characterization of the rice *ssg4* mutant identified a novel factor that influences SG size (Matsushima et al. 2014). In *ssg4* endosperm, the cross-sectional areas occupied by SGs are up to 6-fold greater compared with that of wild-type. SSG4 protein is composed of 2,135 amino acids and contains an N-terminal amyloplast-targeted sequence. SSG4 also contains a DUF490 (Domain of unknown function 490) that is conserved from proteobacteria to higher plants. A Gly → Ser amino acid substitution occurs in *ssg4* DUF490. DUF490-containing proteins with lengths greater than 2,000 amino acid residues are predominant in photosynthetic organisms but are minor in proteobacteria. To date, SSG4 homologues of photosynthetic organisms have not been functionally characterized.

The enlargement of SGs does not result in the direct expansion of starch granules in *ssg4*, because SGs in *ssg4* endosperm are the compound SG type. SSG4 homologues in other crops such as barley, maize, and sorghum have a conserved glycine residue at the *ssg4* mutation site (Matsushima et al. 2014). These crops develop simple SGs; therefore, the size of SG is consistent with the size of starch granules. Introduction of the same Gly → Ser mutation into these crops or downregulation of *SSG4* homologues will achieve engineered starch granule enlargement.

13.9 Future Prospects

Cereal mutants with abnormal SG morphologies are not many. Large-scale screening will isolate novel cereal mutants with altered SG morphologies. Such mutants are expected to provide novel physicochemical properties and tastes. The starch of rice *ae* mutant that is allelic with *ssg1*, *ssg2*, and *ssg3* has low amylase digestibility (Kubo et al. 2010). Some saccharides in *ssg4* are elevated compared with those of wild-type (Matsushima et al. 2014). Collecting and identifying mutants with related SG morphologies is important for future breeding programs to improve grain quality. The rapid SG observation method is an effective strategy for isolating such mutants. Using this strategy, commercially desirable mutants such as glutinous rice (*waxy* rice) and sweet corn can be identified in future. These mutants will also provide greater insight into the molecular mechanisms that determine SG morphologies. Ultimately, this will deepen our understanding of interspecific morphological SG variations and evolution of SG morphology.

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