

## VARIATION OF STARCH GRANULES IN DIPLOID SPECIES OF THE GENUS *AVENA* L.

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**Abstract.** Composite starch granules, the main product of assimilation in oat endosperm, were analyzed in the accessions of both wild and cultivated diploid species of the genus *Avena*. Simple starch granules are mostly synthesized in the outer parts of the endosperm tissue. The size of sub-grains in a composite granule does not depend on a wild or cultivated status of the species. Inter-specific variation in the size differences of composite granules is large. Also, a broad variation has been detected for granules analyzed in a Lugol's solution or polarizing light. This analysis revealed a difference between the synthesis of amylopectin *versus* amylose in a granule. Examples of occurrences of low levels of amylopectin synthesis are provided.

**Key words:** *Avena* diploids, starch granules, variation

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### Introduction

Many studies have shown that the development of endosperm tissue in grass caryopsis occurs according to a clonal pattern. The nature of the tissue in a free nuclear stage has been described as a body composed of sub-syncytial units, that is, groups of nuclei of different origin (KOSINA 2009). For instance, in *Avena strigosa* Schreb., two adjacent cells of endosperm can synthesize starch granules of different size due to their genetic difference. On the other hand, the interior of the cell can differ in starch synthesis in its two regions, central *versus* external, and such a type of cells can create a single clone, e.g. in *A. brevis* Roth (KOSINA 2009).

In wheat, starch granules of three different sizes, A, B and C are synthesized (WILSON *et al.* 2006; KOSINA & TOMASZEWSKA 2011b). The A- and B-granules dominate in the genus *Hordeum* L. (BAUM & BAILEY 1987).

A bimodal size of starch granules (A and B) has been noted for rye, but in millet, rice or triticale ( $\times$  *Triticosecale* Wittm. ex A. Camus) it is unimodal (TESTER *et al.* 2004). Some data on the inter-specific variability of the size of subunits in the *Avena* L. composite starch granules have already been provided by KOSINA (2007).

Changes in the size of starch granules were described by KLEMSDAL *et al.* (1986) in the *Risø* high lysine barley mutants. Granules were small and caryopses poorly filled by endosperm tissue. Other starch mutations such as *Risø 17* and *Notch-2* were studied by BURTON *et al.* (2002). These mutants synthesize phytylglycogen and in their plastids several starch granules develop. In ripe caryopses, atypical starch granules are composite.

Other characteristics of starch granule can be detected by a Lugol's reaction or by imaging a granule in a polarized light. The amylopectin-poor starch granule appears as a Lugol's light

body or is lightly colored when observed under a polarizing microscope. Amylopectin-poor mutations of single starch granules were found in amphiploids such as *Elymus canadensis* L. × *Pseudoroegneria libanotica* (Hack.) D.R. Dewey and *Triticum dicoccum* Schrank ex Schübl. × *Aegilops squarrosa* L. (*A. tauschii* Coss.) or *Leymus racemosus* (Lam.) Tzvelev (KOSINA *et al.* 2015). PATRON *et al.* (2002) described cultivars in waxy barley with a low level or with no amylose starch granules. The level of amylose depends on the activity of granule-bound starch synthase I (GBSSI). Types with low- or free-amylose contents are probably of Chinese origin. Waxy mutants expressing lowered activity of GBSSI have also been obtained in *Avena strigosa* (VERHOEVEN *et al.* 2004). In potato, starch granules with low activity of GBSSI are not stained by Lugol's iodine (EDWARDS *et al.* 2002) and such types have also been documented in some members of Triticeae (KOSINA *et al.* 2015).

### Material and methods

Accessions of the *Avena* diploids were cultivated on small plots under the same soil and climatic conditions in R. Kosina's grass collection. Thus, the study material was treated as originating from a completely randomised one-way classification design. Starch granules of the following species were studied (in brackets, the numbers of accessions are provided): *A. brevis* Roth (4), *A. canariensis* Baum, Rajhathy et Sampson (1), *A. hirtula* Lag. (2), *A. longiglumis* Dur. (5), *A. nuda* L. (1), *A. pilosa* (Roem. et Schult.; syn. *A. eriantha* Dur.) (1), *A. strigosa* Schreb. (34) and *A. wiestii* Steudel (4). From broken caryopses, starch granules were isolated, mounted on slides in glycerin and stained by a Lugol's solution according to BRODA (1971). Non-stained starch granules were observed under a polarizing Amplival microscope and documented by a Zenith TTL camera with Fuji 400 film. Granules with high amounts of

amylopectin stained with Lugol's iodine were dark brown in color, and when observed under a polarizing microscope a distinct, red and blue coloration was noted.

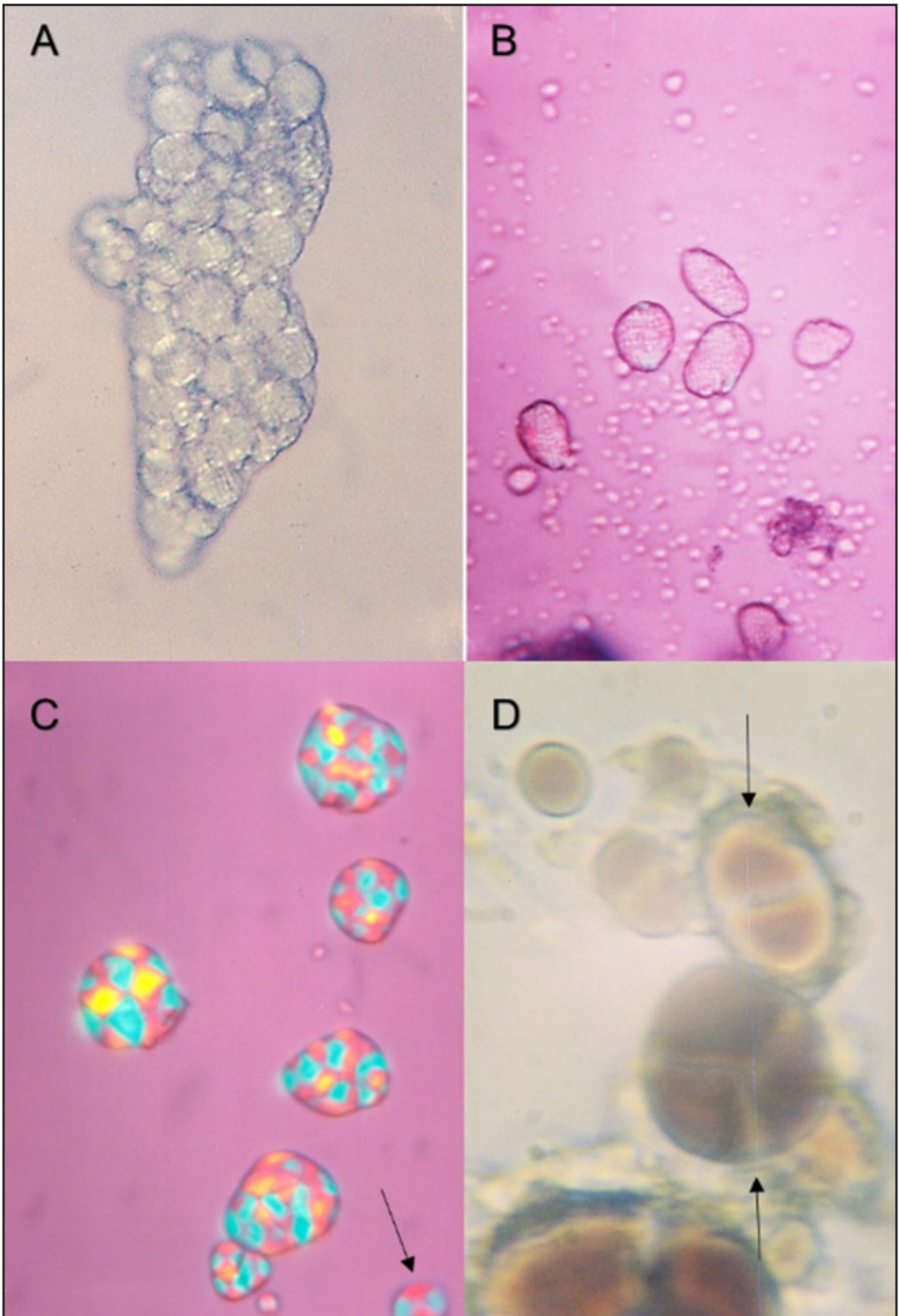
### Results and discussion

In general, composite starch granules are synthesized in the endosperm of oats. However, the synthesis of simple granules adds them to a total starch pool in the tissue. Such dual morphogenesis especially occurs in the outer cells of endosperm which are adjacent to a high-protein (HP) subaleurone layer, *e.g.* in *A. hirtula* (KOSINA 2009). The simple granules are also synthesized and embedded in a protein mass in the HP layer. The size of composite granules appeared highly variable among diploid oat species. This variation is exemplified in Fig. 1. In *A. pilosa* and *A. eriantha*, the granules are very fine-grained and composed of many grains. In *A. pilosa*, fine-grained granules, mainly circular, are embedded in a fatty mass (Fig. 1 A); *A. eriantha* has granules that are elongated and ellipsoidal in shape (Fig. 1 B). Granules in *A. wiestii* are larger and composed of only several starchy units (Fig. 1 C, D). Under polarizing light, they presents strong coloration (Fig. 1 C). This proves that the synthesis of amylopectin in these granules is very effective. *A. canariensis* has granules similar to those in *A. wiestii*. However, granules from another sample in *A. wiestii* differ with respect to amylopectin synthesis (Fig. 1 D). Lighter granules synthesize less amylopectin.

In a cultivated species, *A. strigosa*, fine-grained granules are several times larger than those in *A. eriantha*. A wild species, *A. longiglumis*, expresses a similar Lugol's staining diversity as *A. wiestii* (GRABIŃSKA 2008).

The above examples show that oat diploid species vary with respect to size of sub-grains present in the composite starch granules. Two types of granules are distinguished, coarse-

**Fig. 1.** Composite starch granules: **A** – *Avena pilosa*; **B** – *A. eriantha*; **C, D** – *A. wiestii*. **A-D** are taken under a light microscope; **A-C** under a polarised light; **D** – shows the granules stained in a Lugol's solution. Microscopic magnification: **A-C** – ×320; **D** – ×800.



grained in *A. wiestii* and *A. canariensis* versus fine-grained in *A. strigosa*, *A. pilosa* and *A. eriantha*. Each pool of granules is composed of composite and single ones, and proportion between both probably depends on the age of the tissue and its location in the caryopsis. Granules between each other also differ in the level of amylose versus amylopectin synthesis.

Morphogenesis and metabolic potential of starch granules can be inter- and intra-cellular and intra-granule variable (KOSINA 2009). Such types of variation have also been noted in the endosperm aleurone layer (KOSINA & TOMASZEWSKA 2011a), and create mosaic patterns of the endosperm development (KOSINA 2007). These variations are of mutational origin. Amyloplasts expressing amylose synthesis mutations were detected in barley (PATRON *et al.* 2002), oat (VERHOEVEN *et al.* 2004) and pea (EDWARDS *et al.* 2002).

*A. pilosa* has been considered to be a heterotypic synonym of *A. eriantha* (BAUM 1977). And the differences between both species in endosperm microstructure support such status of *A. pilosa*.

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