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The Distribution Features of Polysaccharides and Lipids in the Development of Tomato Anthers

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Abstract

The regulation of nutrient transportation and transformation in developing anthers is very complex. We analyzed the distribution and features of polysaccharides and lipids in the developing anthers of tomatoes using histochemical methods. Some starches appeared in the connective somatic tissue of anthers during the sporogenous cell stage. Before meiosis of the microspore mother cell, a thick polysaccharide callose wall was formed, accompanied by a reduction in the connective tissue starches. During the tetrad stage after meiosis, the polysaccharide material in the anther did not change. At the early microspore stage, the starches in the connective cells again increased, and polysaccharide material appeared in the partial intine of pollen. At the late microspore stage, a large vacuole formed that did not contain lipids or starches, and only the pollen wall contained red polysaccharides. At this stage, the connective somatic cell starch amounts decreased, and the tapetal cells changed shape and degenerated. After microspore division, abundant lipids appeared in the bicellular pollen, and starches accumulated following pollen development. As the anthers matured, many lipids and some starches accumulated in the epidermal cells. Nutrient metabolism within the tomato pollen characteristically accumulated lipids first and then starches, while the mature pollen accumulated starches and lipids simultaneously. This characteristic pattern of nutrient metabolism in tomato pollen shows species specificity among plants.

Keywords: tomato; anther development; starch; polysaccharide; lipid

1. Introduction

The anthers of angiosperms are the most complex male organ. The anther wall consists of the epidermis, endothecium, middle layer and tapetum. The middle layer and tapetum are the innermost layer of cells and the most closely adjacent, but with different structures and functions. The tapetal cells are of great interest because of their developmental function and close relationship with pollen fertility (Hu, 2005). Pollen development undergoes specialized events, such as meiosis of the microspore mother cell, unequal division of the microspores, generation of the sporopollenin pollen wall and abundant nutrient accumulation in the mature pollen. Although reported to occur early, the regulatory mechanisms for these events is unclear. Nutrient accumulation in mature pollen has family or genus specificity for the timing of starch and lipid deposition.

Lycopersicon esculentum Mill. has a short growth period and small genome, which makes it useful as a research tool in

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classic plant genetics and genome studies (Mohammad, 2011). Basic information on the reproductive biology of *L. esculentum* has been reported (Singh and Brown, 1993), but the nutrient metabolic features of anther development are unknown. Therefore, anther development and the material metabolic features in *L. esculentum* were explored in this study using histochemical methods.

2. Materials and methods

Seeds from *L. esculentum* 'Zhongshu 4' were produced by the Beijing Sihai Seedlings Co. The seeds were grown at the Xiamen University Campus in January 2011 and 2012; the plants bloomed in April. Different sized anthers were collected, squashed and observed under a microscope to determine the exact stage of microsporogenesis. Based on the developmental characteristics of pollen, the anthers were divided into seven stages: sporogenous, microspore mother cell, tetrad, early microspore, late microspore, early bicellular pollen, and mature pollen. All anthers were fixed in 2.5% glutaraldehyde in 0.1 mol · L^{-1} of phosphate buffer (pH 7.2) for 3 h at room temperature, and then washed with buffer three times, for 20 min each. The anthers were post-fixed in 1% OsO_4 in 0.1 mol · L^{-1} of phosphate buffer (pH 7.2) for 15 h at 4 ℃, washed three times in phosphate buffer (pH 7.2), dehydrated in a graded acetone series and then embedded in Epon 812 resin. The resin-embedded anthers were cut into 1 μm sections and attached to slides by heating and drying. Using the methods described by Hu and Xu (1990), the sections were pretreated with 0.5% periodic acid for 10 min and washed for 1–2 min. The pretreated sections were labeled using the periodic acid–Schiff (PAS) reaction for 30 min at room temperature and washed three times for 2 min each in 10% potassium sulfite. Finally, the sections were washed for 5 min in ddH_2O and dried. The PAS detects polysaccharides, which stain a pink/red color. The sections were immersed in to 70% alcohol for 1–2 min and counterstained with 0.3% Sudan black B for 30 min at 60 ℃ to stain the lipids black. After counterstaining, the sections were immersed into 70% alcohol for 1–2 min, washed with ddH_2O and dried. All sections were mounted using glycerin gelatin, and analyzed using a Leica DMR research microscope.

3. Results

3.1. Sporogenous cell stage

The anthers of *L. esculentum* consist of four locules. The anther cell is a rod-like shape before differentiation. Its transection is square and the four corners delineate the four locules. At the sporogenous cell stage, the anthers have several differentiated tissue layers. Listing from the outside to the inside, they are: A layer of epidermis, a layer of endothecium, 3–4 middle layers and an innermost layer of tapetum (Fig. 1, a). All of the cells have vacuoles and a shallow cytoplasmic dye. The epidermal cells are the largest and appear in the outermost layer of the anther. The adjacent endothecium and middle layer cells are smaller than the epidermal cells. At this stage all cells are immature and their types cannot be distinguished. Some starch occurs in the connective cells, but there is little starch in the cells of the anther wall. Sporogenous cells are located in the center of the anther in a horseshoe-shaped arrangement, which is obviously different from the anther wall and connective cells. The cytoplasm of the sporogenous cells are dense and without vacuoles. Its cell walls are thin and have red polysaccharide staining. Neither starches nor lipids are present in the sporogenous cells.

3.2. Microspore mother cell stage

Two changes are evident during the transition from sporogenous cell to microspore mother cell. One is the switch from tightly arranged sporogenous cells to loosely arranged microspore mother cells, which is marked by the development of large intercellular spaces. Another change is the formation of a thick callose cell wall, which is characteristic of microspore mother cells. The anther size increases and the locule space becomes larger during this stage. There are still no starches or lipids to be observed, and only the callose wall displays the red polysaccharide indicator (Fig. 1, b). The cells of the anther wall increase in size and retain a high degree of vacuolization. The tapetal cells also demonstrate high vacuolization. The anther wall cells, located between two locules, do not increase in size and this results in uneven anther walls and dehiscence during anther maturity. There are a few starches in the anther wall cells at this stage but no lipids. The starch in the connective cells decreases compared with the previous stage.

3.3. Microspore tetrad stage

Meiosis is simultaneous in the microspore mother cell of *L. esculentum*. Four microspores in a tetrad arrange into a tetrahedron, surrounded by a polysaccharide positive callose wall. Neither starches nor lipids are present in the cells (Fig. 1, c). There is no evident change in the cells of the epiderm is, endothecium and middle layer. However, the tapetal cells undergo significant changes, including the disappearance of the large vacuoles, decreased cell size and increased cytoplasmic density. Some starch is present in the anther wall and connective cells.

3.4. Early microspore stage

After decomposition of the tetrad callose wall, the four microspores are released and dissociated within the locule. The nucleus of the microspore is in the center of cell, thus the early microspore stage is often named the nucleus center stage. The cytoplasm of the early microspore is dense without evidence of