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Ultrastructural study on dynamics of lipid bodies and plastids during ripening of chili pepper fruits

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ABSTRACT

Dynamics of lipid bodies and plastids in chili pepper fruits during ripening were investigated by means of transmission electron microscopy. Mesocarp of chili pepper fruits consists of collenchyma, normal parenchyma, and huge celled parenchyma. In mature green fruits, plastids contain numerous thylakoids that are well organized into grana in collenchyma, a strikingly huge amount of starch and irregularly organized thylakoids in normal parenchyma, and simple tubes rather than thylakoids in huge celled parenchyma. These morphological features suggest that plastids are chloroplasts in collenchyma, chloroamyloplasts in normal parenchyma, proplastids in huge celled parenchyma. As fruits ripen to red, plastids in all cell types convert to chromoplasts and, concomitantly, lipid bodies accumulate in both cytoplasm and chromoplasts. Cytosolic lipid bodies are lined up in a regular layer adjacent to plasma membrane. The cytosolic lipid body consists of a core surrounded by a membrane. The core is comprised of a more electron-dense central part enclosed by a slightly less electron-dense peripheral layer. Plastidial lipid bodies in collenchyma, normal parenchyma, and endodermis initiate as plastoglobuli, which in turn convert to rod-like structures. Therefore, plastidial lipid bodies are more dynamic than cytosolic lipid bodies. Both cytosolic and plastidial lipid bodies contain rich unsaturated lipids.

1. Introduction

The function of fruits is generally to aid spread seeds. One of the strategies for seed dispersal is that fruits provide attractive nutritious food for animals, which finally pass the intact seeds out of their digestive tubes for subsequent germination. Pepper fruits, which develop beautiful colors to attract birds as they get ripe, are popular cook ingredients and used in food industry (Fernandez-Ronco et al., 2011), for they contain valuable oil and pigments (Tsatsaronis and Kehayoglou, 1971; Perez-Galvez et al., 1999; Schulz et al., 2005; Kwon et al., 2011; Wesołowska et al., 2011).

Unripe pepper fruits generally have a green color, due to the presence of chlorophyll, which characterizes chloroplasts, green plastids. Plastids differentiate into several forms, depending upon which role they play in cells. Few studies have been concerned with ultrastructural dynamics of plastids in pepper fruits before they get ripe.

The ripening process of pepper fruits is typically accompanied by changes in color, from green to red or yellow. The color change is mainly due to the conversion of chloroplasts to chromoplasts (Frey-Wyssling and Kreutzer, 1958; Spurr and Harris, 1968; Laborde and Spurr, 1973). The chromoplast differentiation involves degeneration of chlorophyll, accumulation of carotenoid (Camara and Brangeon, 1981; Whitaker, 1989), metabolism of protein (Siddique et al., 2006; Hadjeb et al., 1988; Newman et al., 1989), and expression of specific genes (Powell and Pryke, 1987; Kuntz et al., 1989; Pozueta-Romero et al., 1997). There have been many researches on ultrastructures of chromoplasts in bell pepper fruits (Frey-Wyssling and Kreutzer, 1958; Spurr and Harris, 1968; Laborde and Spurr, 1973), but very few have been conducted to characterize chromoplasts in chili pepper fruits.

Lipid bodies, also referred to as oil bodies or lipid droplets, are a type of organelles for storage of lipids. The lipid-rich organelles often occur in seeds and fruits in plants (Murphy, 2001). Lipid bodies in plant cells are present in the cytoplasm or within plastids. Those present in cytoplasm are referred to as cytosolic lipid bodies, and those existing within plastids are called plastidial lipid bodies. Knowledge of lipid bodies in fruits and seeds is increasing (Platt-Aloia and Thomson, 1981; Murphy, 2001; Jiang and Tzen, 2010). It is widely known that pepper fruits contain rich lipids (Tsatsaronis and Kehayoglou, 1971; Whitaker, 1989; Perez-Galvez et al., 1999; Schulz et al., 2005; Kwon et al., 2011; Wesołowska et al., 2011). However, little ultrastructural work has been done to characterize lipid bodies in pepper fruits.

Capsicum annuum L. covers a lot of varieties, including bell peppers and chili peppers. 'Hanguohong', an early maturing variety

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of this species, produces horn-shaped fruits, 11–14 cm in length, 1–2 cm in diameter. Fruits are green when unripe and ripen to bright red. Since this variety bears hot fruits that produce burning sensation, it is called chili pepper, in contrast to bell peppers, which produce capsaicinoid-free fruits that are hence not hot.

Therefore, this research is aimed to understand ultrastructural dynamics of lipid bodies and plastids in fruits of this chili pepper during ripening by means of light and transmission electron microscopy.

2. Materials and methods

Seeds of *C. annuum* L. cv. Hanguohong were sown in a nursery bed on 1st of May, 2008, when local average daily temperature rose up above 14 °C. Later, 50 day old seedlings were transferred to well-drained and well-irrigated field of sandy soil and fertilized with composted cattle manure.

Fruits that have reached full size and are still green (mature green) as well as red fruits were collected in late August and early September. Collected fruits were cut into pieces (<1 mm × 1 mm) and were immediately immersed in 2% glutaraldehyde solution (in 0.05 M phosphate buffer, pH 6.8) for primary fixation for 2 h. The secondary fixation was carried out in 1% osmium tetroxide (in the same phosphate buffer) at 4 °C for 2 h in darkness. Dehydration was carried out in 10% upgraded ethanol series. Following that, samples were embedded in Embed-812 resin. 1 μm semithin and 70 nm ultrathin sections were cut on a Leica ultramicrotome. For investigation of anatomical structure of fruit, semithin sections were stained with Toluidine Blue before observation under the light microscope. For cytochemical examination of lipid bodies, semithin sections were directly observed without further staining. The sections for transmission electron microscopy were stained with 5% uranyl acetate followed by lead citrate solution. Observation and photography were performed under a Jeol 1220 and a Philips Tecnai 12 transmission electron microscope. The experimental work was carried out during the years from 2008 to 2010.

3. Results

3.1. Fruit anatomy

Chili pepper fruits consist of exocarp (epidermis), mesocarp, and endocarp (endodermis) (Fig. 1A), which form a locule wherein seeds develop. Both exocarp and endocarp is a single layer of small cells (Fig. 1B and C). Epidermis cells have an extensively thickened outer wall (Fig. 1C). Endodermis cells, which are smaller than epidermis cells, slightly arch toward the locule (Fig. 1B).

Mesocarp, which forms the majority part of the fruit, consists of collenchyma and parenchyma. Collenchyma, which is located subepidermally, includes 6–7 layers of cells that are characterized by thickened walls. Collenchyma cells are obviously smaller and more angular in the 2 outer layers than in the inner layers. Collenchyma cells are closely arranged and thus very few intercellular spaces occur (Fig. 1A and C).

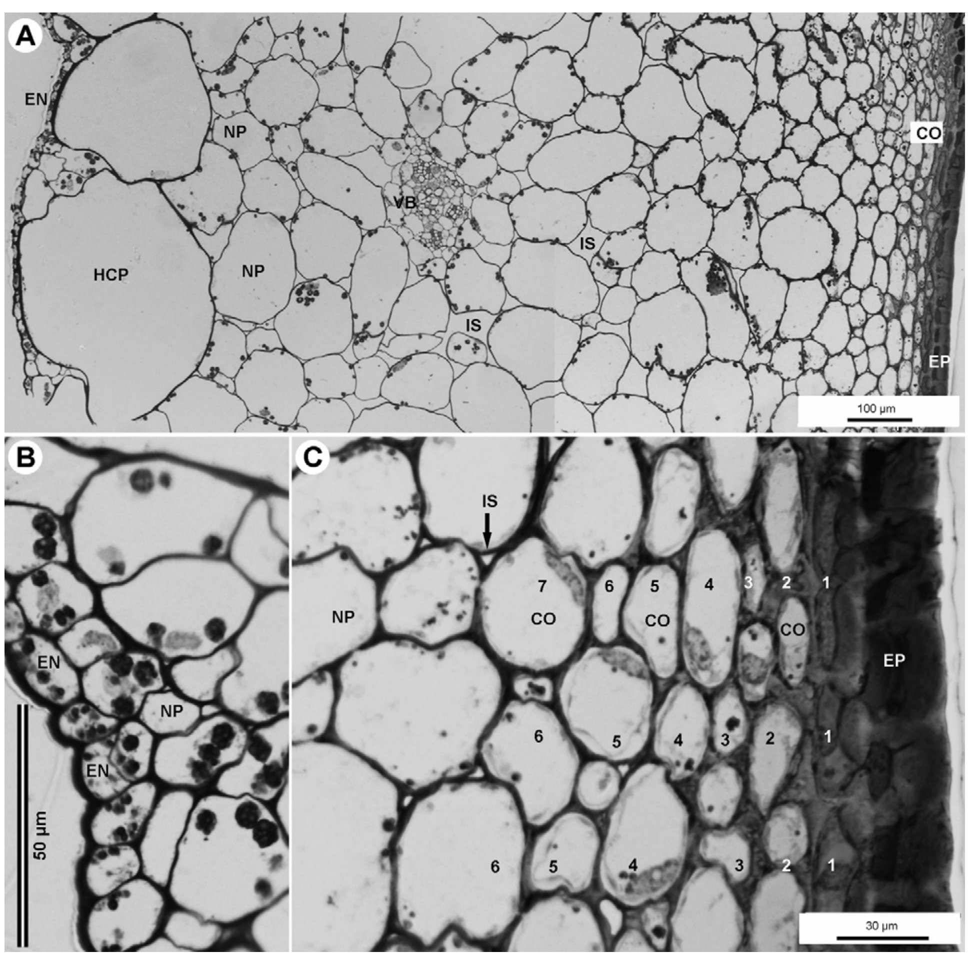

Fig. 1. Anatomical structure of chili pepper fruits. (A) Epidermis, collenchyma, normal parenchyma, huge celled parenchyma, and endodermis. Vascular bundles are buried in normal parenchyma, (B) endodermis, and (C) collenchyma and epidermis. CO, collenchyma; EN, endodermis; EP, epidermis; HCP, huge celled parenchyma; IS, intercellular space; NP, normal parenchyma; VB, vascular bundle.