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Ultramicroscopy reveals that senescence induces in-situ and vacuolar degradation of plastoglobules in aging watermelon leaves

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ABSTRACT

The dynamics of plastoglobules in chloroplasts in aging watermelon leaves were examined by means of transmission electron microscopy, with the aim to understand the intracellular sites for the degradation of plastoglobules in response to leaf senescence. Plastoglobules in chloroplasts in aging leaves with 40% loss of chlorophyll increased drastically in number and size in comparison with young and mature leaves. As senescence advanced, plastoglobules underwent degradation within chloroplasts, or were secreted outside chloroplasts. There were two distinct types of secretion. One type was that chloroplasts protruded to form plastoglobule-containing vesicles and, as the vesicles were detached from chloroplasts, plastoglobules were carried outside chloroplasts. The other type was that plastoglobules squeezed out through the chloroplast envelope into cytoplasm. Lipid droplets were present in the vacuole and underwent degradation therein. Lipid droplets in the vacuole shared similar ultramicroscopic appearance with plastoglobules in chloroplasts, indicating that plastoglobules were engulfed and degraded by the vacuole after they were secreted outside chloroplasts. These results suggested that senescence induces both in-situ and vacuolar degradation of plastoglobules in aging watermelon leaves.

1. Introduction

Chloroplasts contain both membrane lipids and storage lipids. There is a high abundance of membrane lipids, for the chloroplast contains the most abundant cellular membrane system, photosynthetic thylakoids, which function as the carrier of photosystems and electron transport chains and as the major sites of acyl lipid biosynthesis (Murphy, 2001). The storage lipids include a variety of neutral lipids that are often mixed with plastoquinone, tocopherols, and variable amounts of lipophilic pigments, mainly carotenoids. The mixtures of these macromolecules are stored in plastidial lipid droplets, known as plastoglobules (Murphy, 2001, 2012). A plastoglobule is a macromolecular assembly bounded by a polar lipid monolayer membrane associated with a variety of proteins. Plastoglobules in chloroplasts in leaves mainly take a spherical shape (Murphy, 2001), while they may become rod-like in chromoplasts in other parts such as floral nectaries (Liu and Zhao, 2011) and ripe fruits (Simkin et al., 2007; Liu, 2013). Plastoglobules are in close association with thylakoids. There is a permanent structural coupling between plastoglobules and thylakoid membranes (Austin et al., 2006). The components of the plastoglobule, such as neutral lipids, carotenoids, plastoquinone, and tocopherols, are in a dynamic equilibrium with the components of the thylakoid membrane (Murphy, 2012). The dynamic equilibrium is shifted towards plastoglobules in response to the occurrence of leaf senescence, the last stage of leaf development. During leaf senescence, thylakoid membranes tend to be degenerated, producing a large amount of fatty acids, which are in turn synthesized into neutral lipids. These neutral lipids are temporally stored in plastoglobules, leading to a sharp increase of plastoglobules in number and/or size (Kaup et al., 2002; Padham et al., 2007; Fan et al., 2013). These plastoglobules subsequently undergo rapid degradation and the resulting fatty acids are finally converted to phloem-mobile sucrose that is translocated to growing sites (Kaup et al., 2002). A question is: where do these plastoglobules undergo rapid degradation during leaf senescence, within or outside chloroplasts?

Lipases as well as proteases that are up-regulated during leaf senescence are mostly located in the vacuole or other sites outside the chloroplast (Xu et al., 1996; Drake et al., 1996; Buchanan-Wollaston, 1997; Buchanan-Wollaston and Ainsworth, 1997; Nam, 1997; Bhalerao et al., 2003; Buchanan-Wollaston et al., 2003; Gepstein et al., 2003; Guo et al., 2004), indicating the possibility that the rapid degradation of plastoglobules and proteins binding them could occur in the central vacuole or other sites outside the chloroplast. Ultramicroscopic observations have

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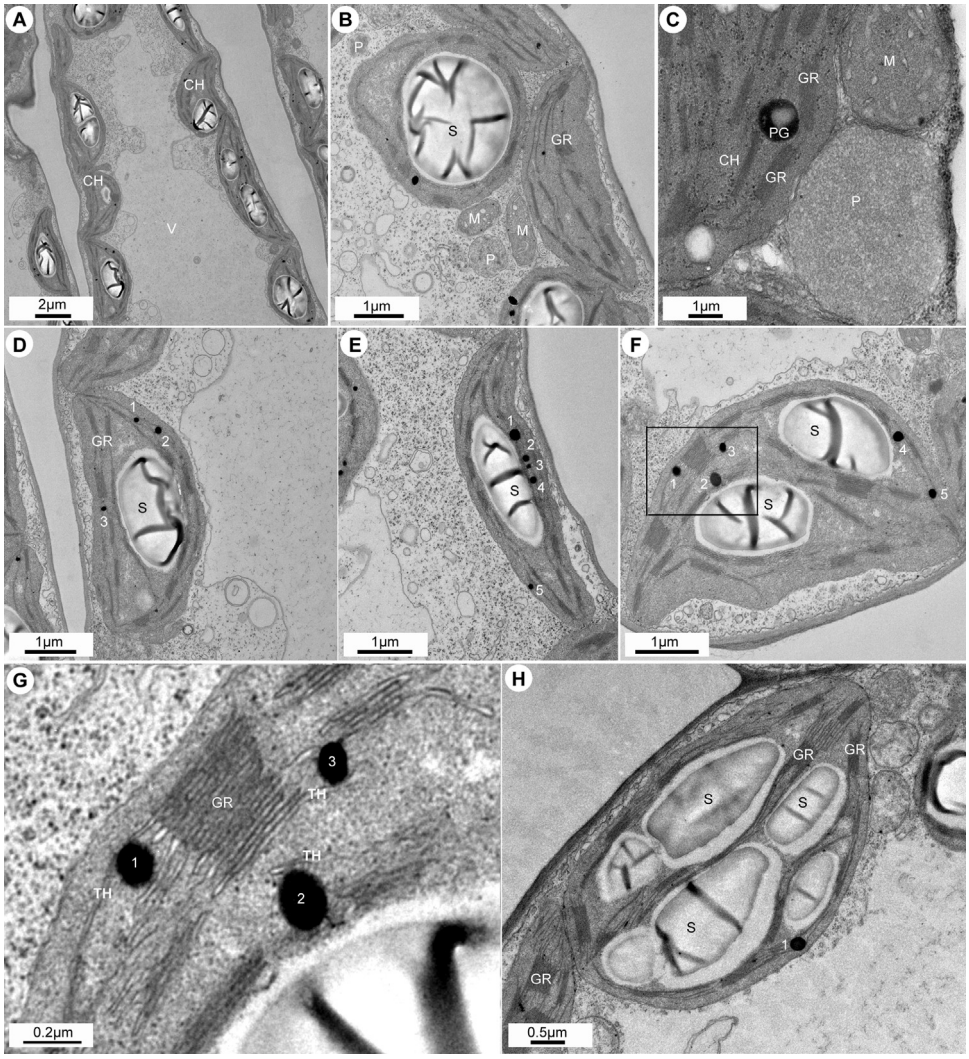

Fig. 1. Chloroplasts in expanding (A–G) and fully expanded (H) leaves of watermelon. (A) Mesophyll cells contained a central vacuole that pressed cytoplasm against the cell wall. There were a large number of chloroplasts in each cell. (B) Chloroplasts with mitochondria and peroxysomes nearby. (C) Close association of chloroplasts, a peroxysome, and a mitochondrion. (D–F) Chloroplasts, showing starch grains and plastoglobules (marked by numbers). (G) Higher magnification of the squared region in F, showing close association of plastoglobules (marked by numbers) with thylakoids. (H) A chloroplast in a fully expanded leaf. CH: chloroplast; GR: grana; M: mitochondrion; P: peroxysome; PG: plastoglobule; S: starch grain; TH: thylakoid; V: vacuole.

demonstrated that plastoglobules are secreted from the chloroplast in senescent leaves (Guiamét et al., 1999), indicating that plastoglobules might undergo degradation outside the chloroplast during leaf senescence. Furthermore, enhancement of autophagy related genes required for nutrient recycling has been demonstrated in senescent leaves (Doelling et al., 2002), suggesting increased activity of autophagy. The observation of engulfment of chloroplasts by the vacuole (Gepstein, 1988; Noodén, 1988) supports the idea of vacuolar degradation of chloroplast components. Therefore, plastoglobules in senescing leaves may undergo degradation within three compartments: chloroplasts, vacuoles, and/or cytoplasm. However, ultramicroscopic observation of degradation of plastoglobules during leaf senescence has been few.

In this research, I examined the intracellular sites for the degradation of plastoglobules in aging leaves of watermelon by means of transmission electron microscopy. The results of this research suggested that plastoglobules in aging watermelon leaves undergo degradation within both chloroplasts and vacuoles, i.e., plastoglobules underwent both in-situ and vacuolar degradation during leaf senescence.

2. Materials and methods

Seeds of watermelon were sown in a nursery bed in a greenhouse in March, 2013 and 2014, when local average daily temperature rose up above 5 °C. A 50 days old seedlings were transplanted in the field fertilized with livestock manure. During June and July, leaf samples of different ages were collected from expanding, fully expanded, and aging leaves with chlorophyll content decreased by 40%. Chlorophyll concentration was determined according to the method of Arnon (1949).

Leaf samples were cut into small pieces (<1 mm × 1 mm) and were then immediately immersed in 2% glutaraldehyde solution (in 0.05 M phosphate buffer, pH 6.8) for primary fixation for 4 h. The secondary fixation was completed in 1% osmium tetroxide (in the same phosphate buffer) for 4 h in darkness. Dehydration was carried out in 10% upgraded ethanol series followed by acetone. After dehydration, samples were embedded in Embed-812 resin and were sectioned on a Leica ultramicrotome at 60 nm thickness. The sections were stained with uranyl acetate followed by lead citrate solution. Observation and photography were performed under a Jeol 1220 transmission electron microscope.