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Lipid globules on the plastid surface in *Iris* tepal epidermis cells during tepal maturation and senescence

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ABSTRACT

Epidermis cells in the outer tepals of *Iris* flowers (*Iris* × *hollandica*, cv. Blue Magic) start programmed cell death (PCD) prior to floral opening. The tepals show visible senescence symptoms three days after full opening. Visible senescence coincides with collapse (death) of the upper epidermis cells. In these cells, electron-dense particles (plastoglobuli), membranes, and oil bodies were observed in the plastid interior. Electron-dense globules similar to plastoglobuli, thus apparently mainly consisting of lipids, were found on the plastid surface, from before flower opening until cell death. Such electron-dense globules were also present in the cytosol. The size of some of the globules on the plastid surface increased with time. The globules are likely involved in transfer of lipidic/proteinaceous material from the plastid to the cytosol. As the plastids contained ample oil bodies, up to the time of cell death, cell death was likely not due to lack of reserves. Mitochondrial ultrastructure also remained the same until cell death. The role of mitochondria in PCD is discussed.

Introduction

The end of the life span of a flower can be due to abscission of the petals, to permanent flower closure and desiccation, and to petal senescence, which becomes visible as withering (wilting) and colour changes. Senescence can be defined as a process observable at the organ level, while the underlying processes at the cellular level is programmed cell death (PCD). We studied senescence/PCD in *Iris* cv. Blue Magic flowers. The flowers have two whorls of blue leaves, called tepals, three in each whorl. The mesophyll cells of tepals in the outer whorl collapsed on day 1 after the flower had opened. Two days later this was followed by collapse of the epidermis cells, which produced the visible senescence symptoms at the organ level, such as a change in tepal colour, and inward rolling at the tepal edges (van Doorn et al., 2003).

Senescence in *Iris* tepals starts early during flower development. Cessation of metabolic coupling and cell–cell signalling via plasmodesmata was associated with senescence. In the epidermis cells this took place before the onset of flower opening. An increase in phospholipase D activity was observed before the end of flower opening (van Doorn et al., 2003).

Chloroplast degradation has become partially elucidated. During senescence most chloroplasts gradually lost their grana and thylakoids, while the number and size of plastoglobuli increased. Major degradation processes inside chloroplasts (Ljubešić, 1968; Butler and Simon, 1971; Hurkman and Kennedy, 1975; Inada et al., 1998; Evans et al., 2010).

Chlorophyll can be catabolized by several plastid-localised enzymes, producing intermediates which are transported to the cytosol and then to the vacuole, but some intact chlorophyll was also found in the cytosol (Hörtensteiner, 2006, 2013). This chlorophyll seems exported from the plastid, at least in part, in bodies having a single limiting membrane (Costa et al., 2013).

Protein degradation also takes place both inside chloroplasts and after transfer to the cytosol and vacuole (Costa et al., 2013; Kato and Sakamoto, 2013). Spherical bodies have been reported to move from the chloroplast to the cytosol. One type of body (called Rubisco containing body, RCB) is limited by a double membrane, while the other (called senescence associated vacuole, SAV) had a single limiting membrane. Both types of bodies contained Rubisco and glutamine synthetase. SAVs contained chlorophyll and thylakoid proteins, while the RCBs contained neither. SAVs reportedly fuse with the vacuole and the contents of the RCBs are also likely deposited in vacuoles (Chiba et al., 2003; Otegui et al., 2005; Ishida

Abbreviations: PCD, programmed cell death; RCB, Rubisco containing body; SAV, senescence-associated vacuole.

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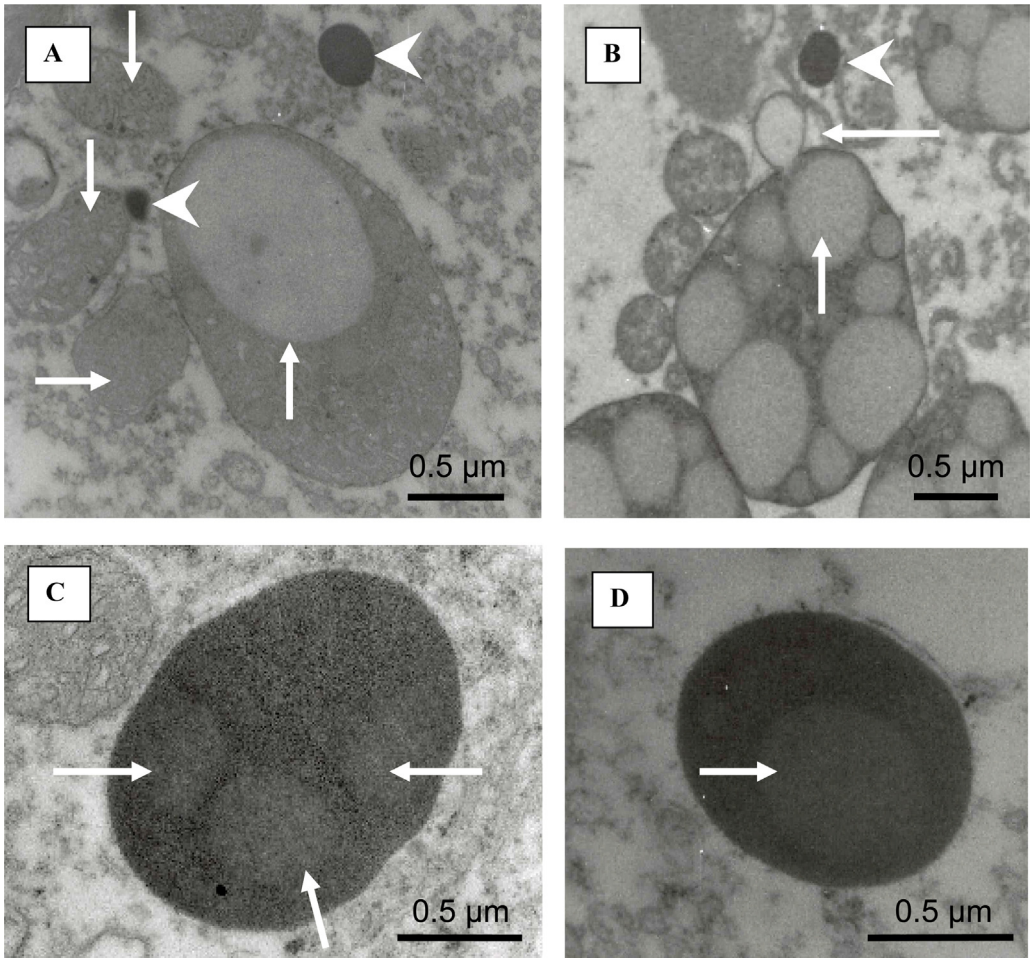

Fig. 1. Organelles in cells at the upper (adaxial) side of the most distal part of the outer tepals, in of *Iris* × *hollandica* cv. Blue Magic flowers that were about to open. (A) Plastid with large body of low electron density (upward pointing arrow), mitochondria (downward pointing arrows), and a peroxisome (arrow pointing to the right). Note bodies with high electron density (arrowheads). (B) Plastid containing relatively large bodies with low electron density (one of which is indicated by an upward pointing arrow). Note protrusion of the plastid (left pointing arrow) and a body with high electron-density (arrowhead). (C) Electron-dense body situated in the cytosol. Note areas in this bodies that are circular or about circular, exhibiting slightly lower electron-density than the rest of the body (arrows). (D) As c, only one area with slightly lower electron-density (arrow). Magnification indicated with bars.

et al., 2008; Martínez et al., 2008; Yamane et al., 2012; Costa et al., 2013; Mulisch and Krupinska, 2013).

Plastoglobuli are bound by a half membrane (single lipid sheath) and contain proteins as well as lipids such as prenylquinones, triacylglycerols, and fatty acid phytyl esters. During senescence, plastoglobule size and number increase due to lipid accumulation. Plastoglobuli are involved in prenylquinone metabolism and likely also in thylakoid disassembly (Besagni and Kessler, 2013). It has been suggested that during chloroplast dismantling plastoglobuli-like (electron-dense) structures are transferred from the plastid to the cytoplasm (Butler, 1967; Mulisch and Krupinska, 2013). For example, in senescing broccoli, small electron-dense globules were observed in the cytosol. These globules were thought to derive from chloroplasts (Terai et al., 2000). Electron-dense globules have been found on the surface of chloroplasts in senescing soybean leaves, which also indicated the possibility of transport of the material from the plastid interior to the cytosol (Guiamét et al., 1999).

Compared to chloroplasts, only little is known about the fate of non-chlorophyll-containing plastids. In tapetum cells of *Tillandsia albida* and *Lobivia rauschii* that were undergoing PCD, such plastids lost starch and membranes while the number of plastoglobuli increased. Later on these plastids disappeared, although possibly only after cell death (Papini et al., 1999, 2011).

We here report on the ultrastructure of epidermis cells in *Iris* tepals, focussing on its plastids. We studied processes related to senescence in tepal epidermis cells, from the time just before flower opening until the time of cell collapse. Electron-dense droplets, very similar the ones found in chloroplasts in senescing leaves, were observed in the plastid interior, at the plastid surface, and in the cytosol.

Materials and methods

Plant material and sampling

Iris flowers (*Iris* × *hollandica*, var. Blue Magic) were obtained from commercial growers in the Netherlands. Flowers were cut at the commercial stage, i.e. with the terminal bud still closed and only its blue tip visible above the sheath leaves. Immediately after harvest, the flowers were placed in water and stored in a 4 °C room until transport to the laboratory the same morning. During transport the stem ends stood in water. Transport took less than 2 h. In the laboratory, the stems were recut in air to a length of 45 cm The stem ends were individually placed in glass vials. Flowers stood in a climate-controlled room at 20 °C, 60% RH, and a photosynthetically active photon flux of 15 μmol m^{-2} s^{-1} (Philips TDL 36W/84 cool white fluorescent tubes) from 7 a.m. to 7 p.m. Samples about