Bangladesh J. Bot. **47**(1): 147-150, 2018 (March) **- Short communication**

CHANGES OF STARCH GRAINS AND PLASTOGLOBULI IN CHLOROPLASTS OF MESOPHYLL CELLS IN *GINKGO BILOBA* L. LEAVES

XIAN-SONG YANG[1,2]*

College of Food and Bio-engineering, Bengbu University, Bengbu 233030, P.R. China

Keywords: *Ginkgo biloba*, Starch grains, Plastoglobuli

Abstract

Observations were made on chloroplasts of mesophyll cells of the newly and expanded young leaves of ginkgo (*Ginkgo biloba* L.) cv. 'Qixingguo' in spring, of the mature leaves in summer, and of the senescing leaves in autumn by transmission electron microscopy. The results showed that there were no starch grains and plastoglobuli in chloroplasts of newly leaves in spring, and there were a small amount of starch grains but no plastoglobuli in expanded young leaves. There were lots of starch grains but only a few plastoglobuli in chloroplasts of mature leaves in summer. The starch grains reduced and disappeared finally but the plastoglobuli increased significantly in chloroplasts of senescing leaves in autumn. The deterioration of the chloroplasts may have relationship between the starch grains decrease and plastoglobuli increase.

Ginkgo biloba L., also known as maidenhair tree, is a well-known living fossil gymnosperm (Major 1967) with edible seeds, medicinal efficacy (Ahlemeyer and Krieglstein 2003), and ornamental value, and is the only representative of the Ginkgoaceae family (Yang and Chen 2014). Such unique characteristics of ginkgo have attracted worldwide interest in plant science research. The genomics (Lin *et al.* 2011, Mohanta 2012), transcriptome (He *et al.* 2016) and the physiology (Skribanek *et al.* 2008, Oukarrouma *et al.* 2016) of ginkgo have been thoroughly investigated. Previous research showed that photo-protection was significantly strengthened at the early stages of leaf expansion in ginkgo in the field and photosynthetic decline in ginkgo during leaf senescence (Yang *et al.* 2012, 2013). However, there have been no studies focusing on changes of starch grains and plastoglobuli in chloroplasts of mesophyll cells in ginkgo leaves.

Ginkgo's leaf emergence in early April, leaf expansion in April to June and full leaf expansion in June, and yellow leaf emergence in late October, is a slow growing deciduous tree. 'Qixingguo' is one of the most popular ginkgo cultivars grown in Jiangsu Province, China (Guo 1993). Ten-year-old female ginkgo plants cultivar 'Qixingguo' were grown in field situated in Jiangdu, Jiangsu Province, P.R. China (32°26′N, 119°38′E). They received standard horticultural practices, diseases and pest control.

Leaf samples were collected at 08:00 in early and late April, late June and late October, respectively, and immediately frozen in liquid nitrogen, and stored at –80ºC until analysis. The fresh leaves were washed with distilled water and the petioles were removed. The middle part of leaves was used and cut into small pieces (about 0.1 × 0.5 cm^2). These small pieces were fixed in a bottle for 2 hrs in 10 cm^3 of 4% (v/v) glutaraldehyde in 0.3 M sodium phosphate buffer (pH 7.5) and the air was pumped out of the bottle with a syringe. The samples were then rinsed and post-fixed for 24 hrs at room temperature in 10 cm^3 of 1.0% (v/v) osmium tetroxide with the same buffer. The post-fixed samples were dehydrated in a graded series of acetone solutions (30, 50, 70, 80 and 90%; 15 min each) and in 100% alcohol (three times by 7 - 8 min), and embedded in

*Author for correspondence: <yangxs2002@sina.com>. [1]College of Life Science, Nanjing Normal University, Nanjing 210023, P.R. China; [2]College of Life Science, Hunan Normal University, Changsha 410006, P.R. China.

epoxy resin mixture. Ultra-thin sections (80 nm) were obtained using a LKB-V ultramicrotome (LKB, Bromma, Sweden) and were collected on copper grids (300 mesh), then stained with 1.0% (m/v) uranyl acetate followed by 5.0% (m/v) lead citrate. Sections were observed at 80 kV using a H7650 (Hitachi, Tokyo, Japan) transmission electron microscope.

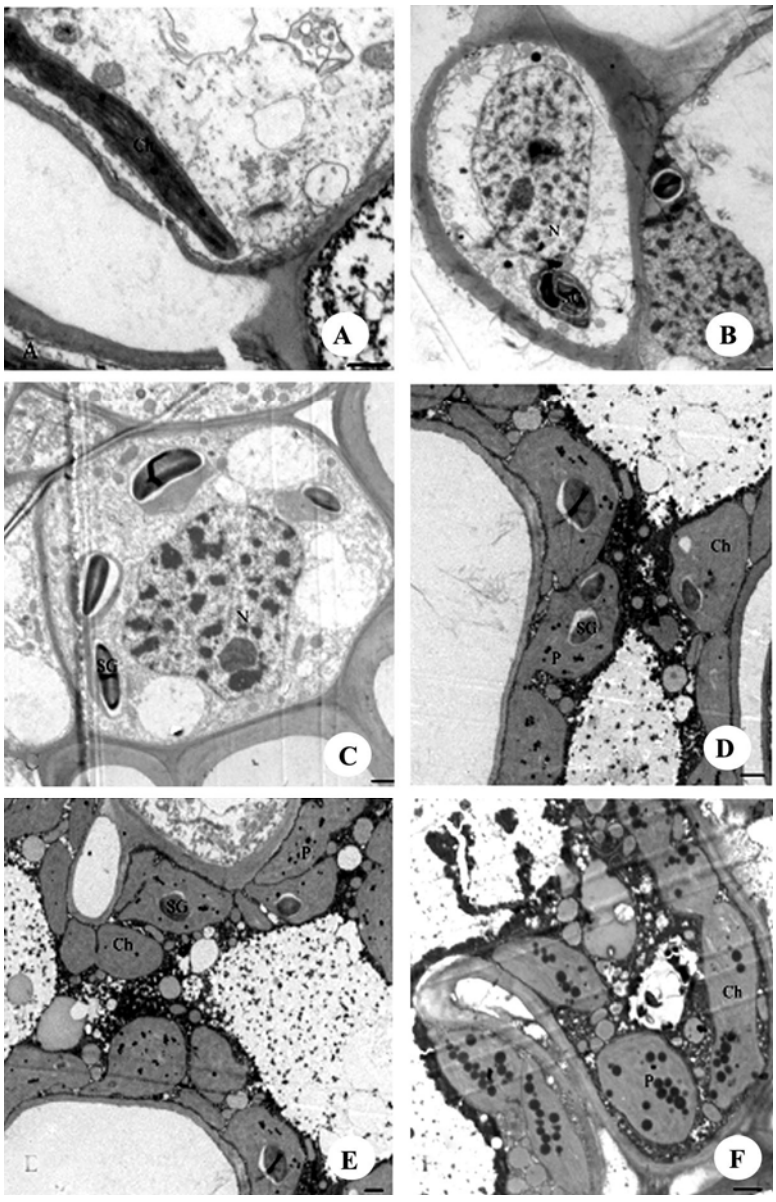

Fig. 1. Changes of starch grains and plastoglobuli in chloroplasts of mesophyll cells in the newly leaves (A) and expanded young leaves (B and C) of ginkgo in spring, of the mature leaves in summer (D and E), and of the senescing leaves in autumn (F). N = Nucleus, Ch = Chloroplast, SG = Starch grain, P = Plastoglobuli. Bars = 1 μm.

Chloroplasts are the sites of photosynthesis and it's structure determines photosynthetic capacity of leaf cells (Yang *et al.* 2013). There were no starch grains and plastoglobuli in chloroplasts of newly leaves in spring (Fig. 1A), and there was a small amount of starch grains but no plastoglobuli in expanded young leaves (Fig. 1B, C). Large plastoglobuli within the chloroplast is the most conspicuous indicator of leaf senescence (Bulter and Simon 1971). In the present study, there were lots of starch grains but only a few plastoglobuli in chloroplasts of mature leaves in summer (Fig. 1D, E). The appearance of plastoglobuli in mature leaves indicated that the leaves began to senesce at this stage. In several studies, more and larger plastoglobuli were observed in different plants with the leaf senescence (Ghosh *et al.* 2001, Riikonen *et al.* 2003, Kivimäenpää and Sutinen 2007). In the present study, the starch grains reduced and disappeared finally but the plastoglobuli increased significantly in chloroplasts of aging leaves in autumn (Fig.1F). For trees and other perennial plants, senescence is illustrated by the splendid colours of autumn. The earliest and most significant change in cell structure is the breakdown of the chloroplast (Lim *et al.* 2007), and the rapid loss of chlorophyll. These changes have been widely used as an indicator of leaf senescence. The deterioration of the chloroplasts might have relationship between the starch grains decrease and plastoglobuli increase.

Acknowledgements

This work was supported by Natural Science Foundation of Anhui Provincial Educational Department (KJ2016A454), State Key Laboratory of Plant Physiology and Biochemistry (SKLPPBKF1502) and Excellent Talents Aid Plan of Anhui Provincial Educational Department (gxfxZD2016273).

References

Ahlemeyer B and Krieglstein J 2003. Neuroprotective effects of *Ginkgo biloba* extract. Cell. Mol. Life Sci. **60**: 1779-1792.

Bulter RD and Simon EW 1971. Ultrastructure aspects of senescence. Adv. Gerontol. Res. **3**: 73-129.

Ghosh S, Mahoney SR, Penterman JN, Peirson D and Dumbroff EB 2001. Ultrastructural and biochemical changes in chloroplasts during *Brassica napus* senescence. Plant Physiol. Biochem. **39**: 777-784.

Guo SJ (ed) 1993. China fruit records: Ginkgo Volume. Beijing, Chinese Forestry Press. pp. 97-98. (In Chinese).

He B, Dong H, Jiang C, Cao FL, Tao ST and Xu LA 2016. Analysis of codon usage patterns in *Ginkgo biloba* reveals codon usage tendency from A/U-ending to G/C-ending. Scientific Reports. **6**: DOI: 10.1038/srep35927.

Kivimäenpää M and Sutinen S 2007. Microscopic structure of Scots pine (*Pinus sylvestris* L.) needles during aging and autumnal senescence. Trees - Structure and Function **21**: 645-659.

Lim PO, Kim HJ and Nam HG 2007. Leaf senescence. Annu. Rev. Plant Biol. **58**: 115-136.

Lin X, Zhang J, Li Y, Luo H, Wu Q, Sun C, Song J, Li X, Wei J, Lu A, Qian Z, Khan I and Chen S 2011. Functional genomics of living fossil tree, *Ginkgo*, based on next-generation sequencing technology. Physiol. Planta. **143**: 207-218.

Major RT 1967. The ginkgo, the most ancient living tree: the resistance of *Ginkgo biloba* L. to pests accounts in part for the longevity of this species. Science **157**: 1270-1273.

Mohanta TK 2012. Advances in *Ginkgo biloba* research: Genomics and metabolomics perspectives. Afr. J. Biotechnol. **11**(93): 15936-15944.

Oukarrouma A, Gharousa ME, Goltsevc V and Strasser RJ 2016. Delayed fluorescence emission as a probe for the response of photosynthetic organisms to high temperature exposure: A comparative study. J. Luminescence **180**: 321-327.

Riikonen J, Oksanen E, Peltonen P, Holopainen T and Vapaavuori E 2003. Seasonal variation in physiological characteristics of two silver birch clones in the field. Can. J. For. Res. **33**: 2164-2176.

Skribanek A, Solvmosi K, Hideg É and Böddi B 2008. Light and temperature regulation of greening in dark-grown ginkgo. Physiol. Plant. **134**:649-659.

Yang XS and Chen GX 2014. Stimulation of photosynthetic characteristics of *Ginkgo biloba* L. during leaf growth. Bangladesh J. Bot. **43**: 73-77.

Yang XS, Chen GX and Yuan ZY 2013. Photosynthetic decline in ginkgo leaves during natural senescence. Pak. J. Bot. **45**: 1537-1540.

Yang XS, Chen, GX, Wei XD and Xie KB 2012. Enhanced antioxidant protection at the early stages of leaf expansion in ginkgo under natural environmental conditions. Biol. Plant. **56**: 181-186.

(Manuscript received on 22 January, 2017; revised on 1 September, 2017)