Pak. J. Bot., 45(5): 1537-1540, 2013.

PHOTOSYNTHETIC DECLINE IN GINKGO LEAVES DURING NATURAL SENESCENCE

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Abstract

Photosynthetic pigments, biochemical activities and chloroplast ultra-structure were investigated to survey the photosynthetic characteristics of ginkgo (*Ginkgo biloba* L.) leaves during natural senescence. During leaf senescence, the content of total chlorophyll decreased rapidly while the content of carotenoids changed little; the content of ATP, the activity of O_2 evolution of chloroplasts, the electron transport activities, the activity of photo-phosphorylation and the activity of Ca^{2+}-ATPase declined; the chloroplasts had damaged in membranes and fewer grana with loose stacks of thylakoids and more and larger osmiophilic granules. With the leaf senescence, the whole structure of chloroplasts became disorganized, the thylakoid membranes became disrupted, and chloroplast was completely disintegrated finally. In conclusion, photosynthetic abilities gradually decreased as the leaves senesced.

Introduction

Leaf senescence is a sequence of biochemical and physiological events that constitute the final stage of plant development. The most remarkable events in leaf senescence are the loss of chlorophyll and the disassembly of the photosynthetic apparatus, which result in decreases in the photosynthetic energy conversion capacity and efficiency. The decrease in electron transport along PS II may be due to an inactivation of the oxygen evolution system or of the PS II reaction center complex, as well as to the inhibition of energy transfer from carotenoids to chlorophyll (Lu *et al*., 2002).

Ginkgo biloba L., is the most ancient living gymnosperm, and is the only representative of the Ginkgoaceae family. Such unique characteristics of ginkgo have attracted worldwide interest in plant science research. Our previous research showed that photo-protection was significantly strengthened at the early stages of leaf expansion in ginkgo under natural environmental conditions (Yang *et al*., 2012). Although changes in the activities of antioxidant enzymes during senescence have been reported (Kukavica & Jovanovic, 2004), as yet there have been no studies focusing on the analysis of leaf photosynthetic and physiological traits of ginkgo during leaf senescence.

In the present study, the changes in photosynthetic pigments, biochemical activities and chloroplast ultra-structure were investigated to survey the photosynthetic characteristics of ginkgo leaves during natural senescence.

Materials and Methods

Plant material: Ten-year-old male ginkgo (*Ginkgo biloba* L.) plants cultivar 'Dafozhi' were grown in field situated in Jiangdu, Jiangsu Province, P.R. China (32°26′N, 119°38′E).

Photosynthetic pigments: Leaf samples were collected at 08:00, immediately frozen in liquid nitrogen, and stored at -80ºC until analysis. The fresh leaves were washed with distilled water and the petioles removed. The samples were extracted in ice-cold 80% acetone, and the extract was centrifuged at 6000 × *g* for 10 min. After collecting the top solution, the precipitate was supplemented with ice-cold 80% acetone, and centrifuged again for another 10 min. The supernatant was measured with a *UV-754* spectrophotometer (Jinpeng Analytical Instruments Co., Ltd., Shanghai, China) at 470, 645 and 663 nm. Chlorophyll contents were calculated as described by Arnon (1949), Ozdener *et al*., (2011), Wang & Chen (2013) and Borzouei *et al*., (2013) and total carotenoids according to Lichtenthaler (1987).

Photochemical functions: Isolation of chloroplasts was performed according to the method of Chen *et al.,* (2004) with slight modifications. Photophosphorylation activity was measured with a luminescent photometer (FG-300, Shanghai Institute of Plant Physiology, Shanghai, China), as described by Yang *et al.,* (2010). The activity of Ca^{2+}-ATPase was measured according to Vallejos *et al*., (1983). An oxygen electrode (Hansatech instruments, UK) attached with a logger was used to measure the activity of photosynthetic oxygen evolution according to Yang *et al.,* (2010). The ATP content was measured by the bioluminescence method described by Zhu *et al*., (2001). Thylakoid membranes were isolated as described by Zhang *et al.,* (2007), with some modifications. The electron transport activities of PS I, PS II, and the whole photosynthetic chain were measured polarographically using a Clark-type liquid-phase electrode (Chlorolab-2; Hansatech, Cambridge, UK).

Transmission electron microscopy (TEM): 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 h 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 h 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 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.

Statistical analysis: All experiments were repeated three times. The results were tested with *SPSS 17.0* for *Windows (SPSS Inc*., Chicago, IL, USA*)* by one-way analysis of variance (*ANOVA*) using Tukey's test calculating at $p<0.05$.

Results and Discussion

Chlorophyll, as a light-harvesting molecule, is a prime component of the photosynthetic system. It is continuously synthesized and degraded as the leaves develop (Thomas, 1997). For trees and other perennial plants, senescence is illustrated by the splendid colors of autumn. The changes in chlorophyll and total carotenoids during leaf senescence were depicted in Fig. 1. The content of chlorophyll *a* and *b* per leaf fresh weight decreased significantly while total carotenoids had no significant changes with the progress of leaf senescence. Chlorophyll content decreased at a faster rate than that of chlorophyll *a* and *b* and the content of chlorophyll *a* is higher than that of chlorophyll *b* (Fig. 1). The content of ATP, the activity of photosynthetic O_2 evolution, photophosphorylation, Ca^{2+}-ATPase (Fig. 2) and the electron transport activities (Fig. 3) declined as the leaf senesced. In the present research, the decrease of chlorophyll content (including chlorophyll a and b) with the process of leaf senescence indicated a gradual damage of photosynthetic apparatus (Fig. 1).

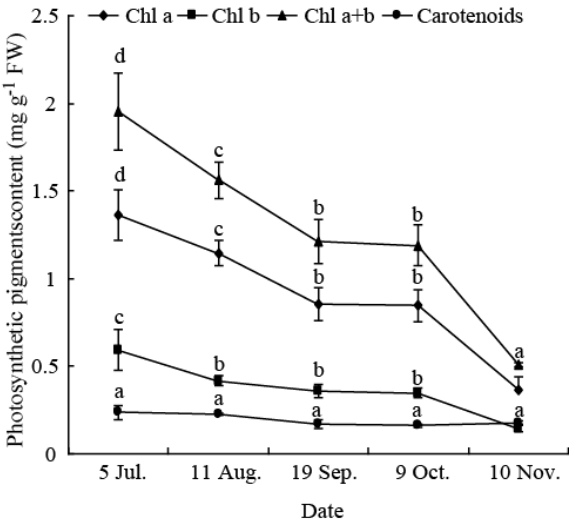

Fig. 1. Changes in the contents of photosynthetic pigments of ginkgo leaves during senescence.

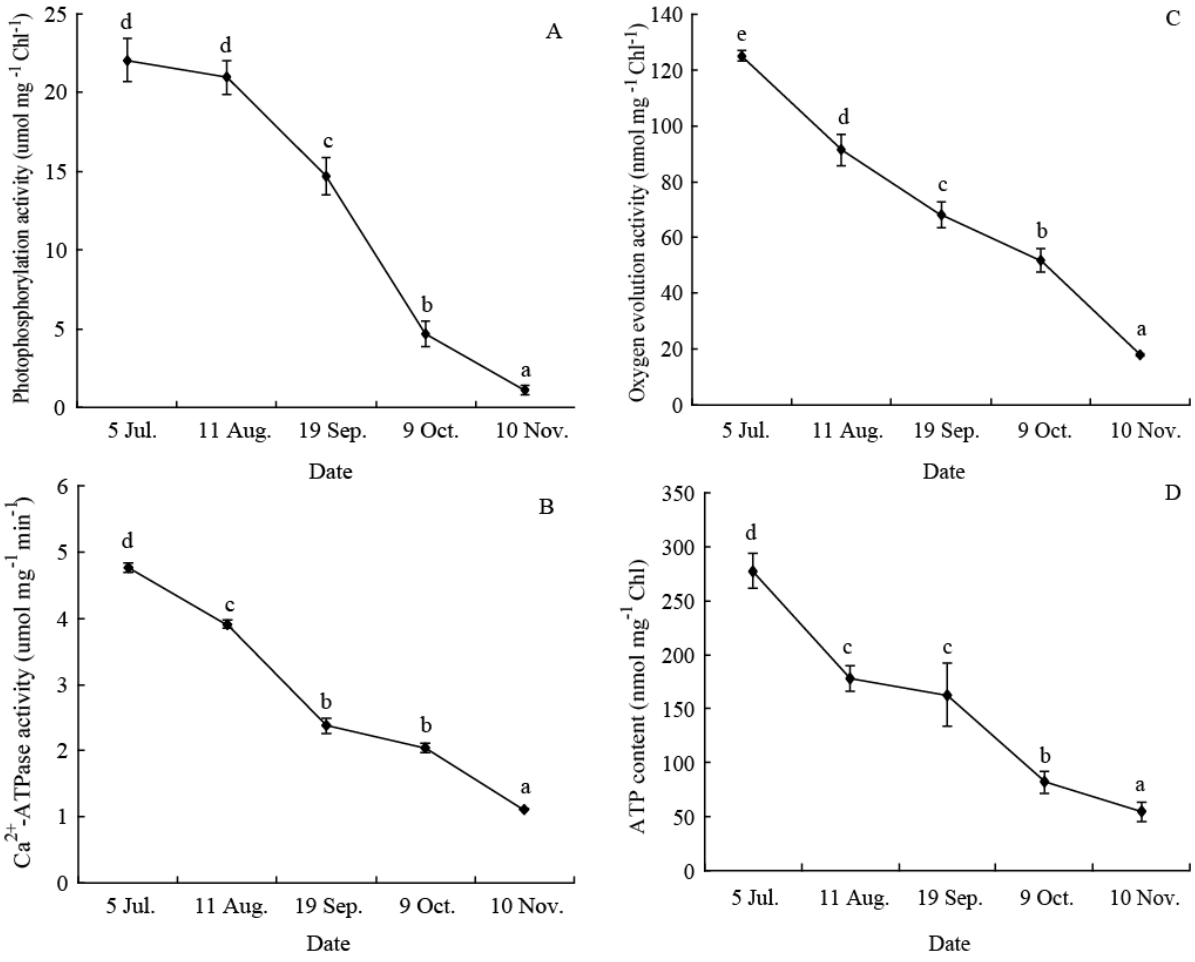

Fig. 2. Changes in the activities of photophosphorylation (A), Ca^{2+}-ATPase (B), photosynthetic oxygen evolution (C) and ATP content (D) of ginkgo leaves during senescence.

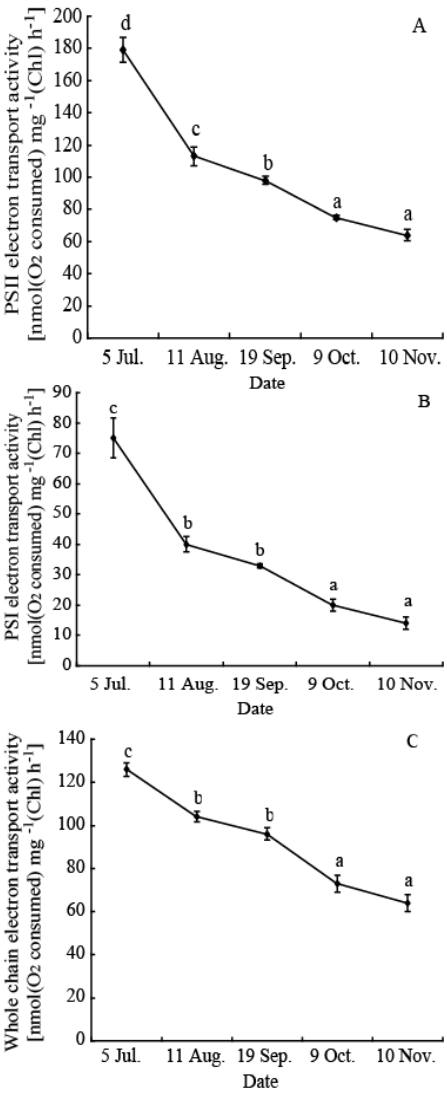

Fig. 3. Changes in the electron transport activities of PS II (A), PS I (B) and whole photosynthetic chain (C) of ginkgo leaves during senescence.

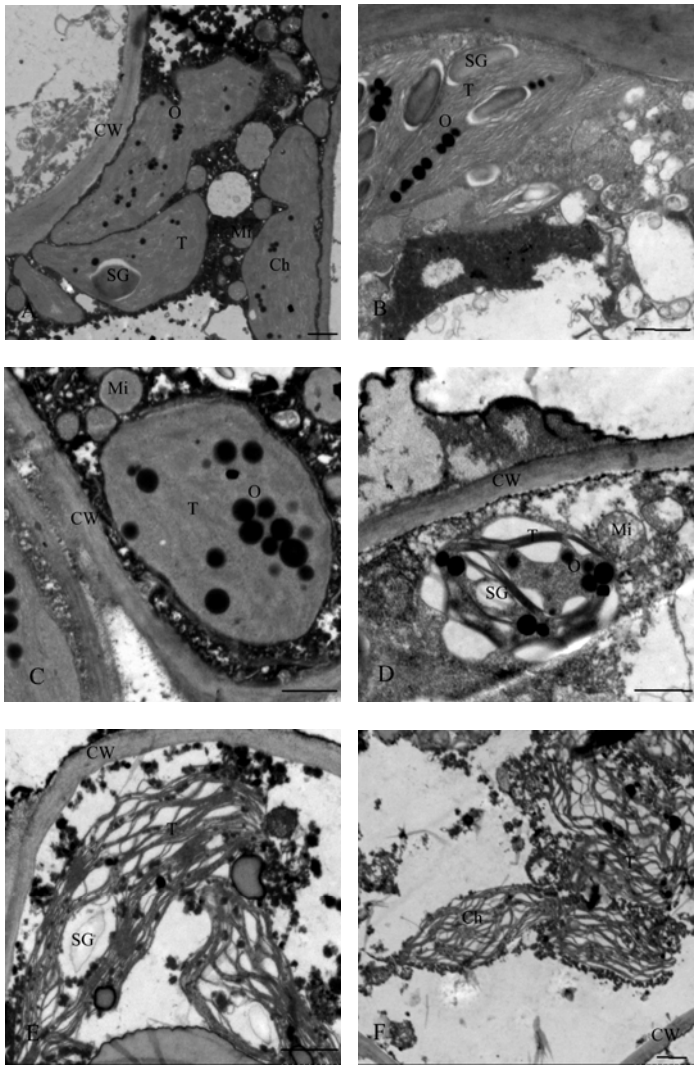

Fig. 4. Changes in the ultrastructure of chloroplasts in ginkgo leaves during senescence on 5 July (A), 11 August (B), 19 September (C), 9 October (D) and 10 November (E and F). CW= cell wall, N= nucleus, Ch = chloroplast, Mi= mitochondria, SG= starch grain, T= thylakoid, O= osmiophilic granule. Bars= 1 μm.

The earliest and most significant change in cell structure is the breakdown of the chloroplast and the rapid loss of chlorophyll (Karatas *et al.,* 2010). Large osmiophilic granules within the chloroplast are the most conspicuous indicator of leaf senescence (Yang *et al.,* 2010). The chloroplast ultra-structure changed significantly during leaf senescence (Fig. 4). As the leaf senesced, the volume of chloroplasts became swollen, the envelope membranes became disrupted, the inclusions became reduced and the grannal thylakoids became loose. The chloroplasts changed from ellipses or ship-like shapes to the irregular circles, had damaged in membranes and fewer grana with loose stacks of thylakoids and more and larger osmiophilic granules. With the leaf senescence, the whole structure of chloroplasts became disorganized, the thylakoid membranes became disrupted, the stroma in chloroplasts almost lost, finally, the chloroplast was completely disintegrated. Chloroplast structure determines photosynthetic capacity of leaf cells. Chloroplasts are the sites of photosynthesis, and the decrease in photosynthesis is expected to correspond with ultrastructural alterations in the chloroplast during leaf senescence (Fig. 4). Therefore, altered thylakoid membrane structure may directly affect membrane functionality and could have deleterious effects on photosynthetic activities of chloroplast, which was supported by declines in photochemical functions of chloroplast such as activities of photophosphorylation (Fig. 2A), Ca^{2+}-ATPase (Fig. 2B), photosynthetic O_2 evolution (Fig. 2C), and the electron transport (Fig. 3).

In summary, the present study showed that changes in the biochemistry of photosynthesis were associated with changes in the ultra-structure of the chloroplasts. Photosynthetic abilities gradually decreased as the leaves senesced.

Acknowledgements

This work was supported by Anhui Provincial Natural Science Foundation (11040606M79) and Natural Science Project of Anhui Educational Department (KJ2010B108).

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(Received for publication 18 April 2012)