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CHANGES IN PHYSIOLOGICAL CHARACTERISTICS OF GINKGO (*GINKGO BILOBA* L.) LEAVES DURING NATURAL YELLOWING

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Abstract

The content of chlorophyll and carotenoids, net photosynthetic rate (P_N), maximum quantum yield of photosystem (PS) 2 photochemistry (F_v / F_m), the activity of superoxide dismutase (SOD) and the content of malondialdehyde (MDA) were investigated to survey the physiological characteristics of ginkgo (*Ginkgo biloba* L.) leaves during natural yellowing. The results showed that the content of total chlorophyll decreased rapidly while the content of carotenoids changed little during leaf natural yellowing, the P_N decreased rapidly while the F_v / F_m changed little during leaf natural yellowing, and the activities of SOD decreased while the content of MDA increased during leaf natural yellowing. Photosynthetic abilities gradually decreased as the leaves yellowing.

Leaves are the main organs for photosynthesis in plants, and the changes in the color of leaves have a direct impact on plant photosynthesis. The main determining factors of the color of leaves in evergreen plants are usually the content of chlorophyll and carotenoids in the leaves. Leaf yellowing is mainly caused by the inevitable influence of chlorophyll synthesis or degradation, which changes the ratio of chlorophyll and carotenoids and exhibits abnormal chlorosis (Yang *et al.* 2021).

Ginkgo biloba L. is the most ancient living gymnosperm, and is an important ornamental tree widely cultivated in China. The ginkgo leaves emerge in early April, then expand from April to June, and turn yellow in autumn. Ginkgo have attracted worldwide interest in biomedical and plant science research (Luo 2025). 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), as yet there have been no studies focusing on the analysis of leaf physiological traits of ginkgo during leaf yellowing.

In the present study, the changes in the content of chlorophyll and carotenoids, net photosynthetic rate, maximum quantum yield of photosystem (PS) 2 photochemistry, superoxide dismutase (SOD) activity and the content of malondialdehyde (MDA) were investigated to survey the physiological characteristics of ginkgo leaves during yellowing.

Ginkgo plants were grown in field situated in Bengbu University, Anhui Province, P.R. China (32°89′N, 117°42′E). Three trees were sampled from 29 September, 2024 (close to the time of leaf yellowing) through 18 November, 2024 (close to the time of leaf drop) on sunny mornings every 10 d. The leaves used for analyses were fully expanded and from lateral branches of the outer part of the crowns with the same exposure to light.

Leaf samples were collected at 08:00 am, 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), and total carotenoids according to Lichtenthaler (1987).

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Photosynthesis rate (P_N) was measured at ambient temperature and at ambient CO_2 concentration (350 μmol mol^{-1}) with a portable photosynthetic system (CIRAS-2, PP Systems, UK). Measurements were performed at 8:00 a.m., when the photosynthetic photon flux density (PPFD) was controlled at 1000 μmol m^{-1}/s *via* an automatic light unit of the CIRAS-2 photosynthetic system. *In vivo* chlorophyll fluorescence was measured using a Handy-PEA chlorophyll fluorometer (Handy-Plant Efficiency Analyser, Hansatech Instruments Ltd., King's Lynn, Norfolk, UK). The transient was induced by red light of about 3,000 μmol m^{-1}/s provided by an array of three light-emitting diodes (peak 650 nm), which focused on the leaf surface to give homogenous illumination over the exposed area of the leaf. The maximum PS 2 quantum yield (F_v / F_m) was determined in dark-adapted (20 min) leaves at 8:00 a.m. according to Strasser *et al.* (1995).

Leaves at different yellowing stages (0.5 g fresh weight) were homogenized under ice-cold condition with 5 cm^3 of 50 mM phosphate buffer (pH 7.0), 10 mM AsA and 1.0% (w/v) polyvinylpyrrolidone. The homogenate was centrifuged at 20,000 × g for 30 min, and the supernatant was collected for enzyme assays. Total SOD activity was assayed by monitoring inhibition of photochemical reduction of nitro-blue tetrazolium according to Giannopolitis and Ries (1977). The activities of SOD were measured using UV-754 spectrophotometer (Shanghai, China). The level of MDA production was assayed to estimate lipid peroxidation according to the method described by Zhao and Li (1999). The MDA in the supernatant was considered to be a thiobarbituric acid-reactive substance. The absorbance was recorded at 532, 600, and 450 nm.

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 $P < 0.05$.

Chlorophyll is continuously synthesized and degraded as the leaves develop (Thomas 1997). The earliest and most significant change in cell structure is the rapid loss of chlorophyll. The changes in chlorophyll and total carotenoids during leaf yellowing were depicted in Figs 1 and 2. The content of chlorophyll per leaf fresh weight decreased significantly while total carotenoids had no significant changes with the progress of leaf yellowing. In the present research, the decrease of chlorophyll content with the process of leaf yellowing indicated a gradual damage of photosynthetic apparatus.

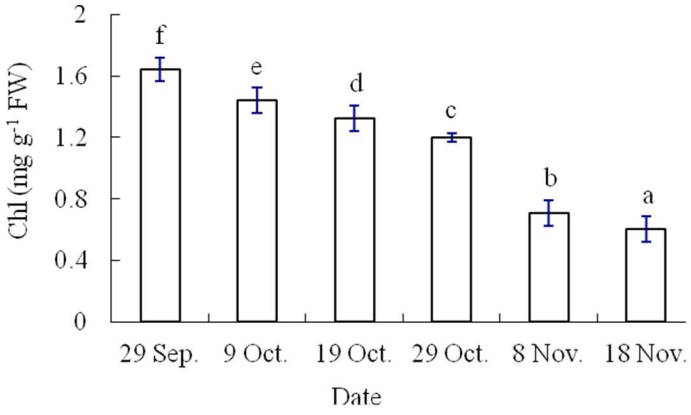

Fig. 1. Changes in the contents of total chlorophyll of ginkgo leaves during natural yellowing.

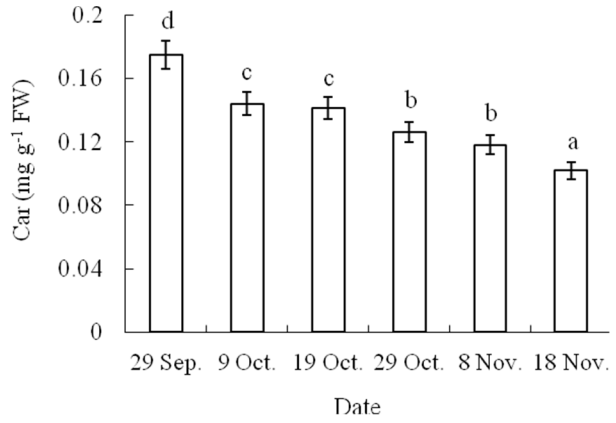

Fig. 2. Changes in the contents of carotenoids of ginkgo leaves during natural yellowing.

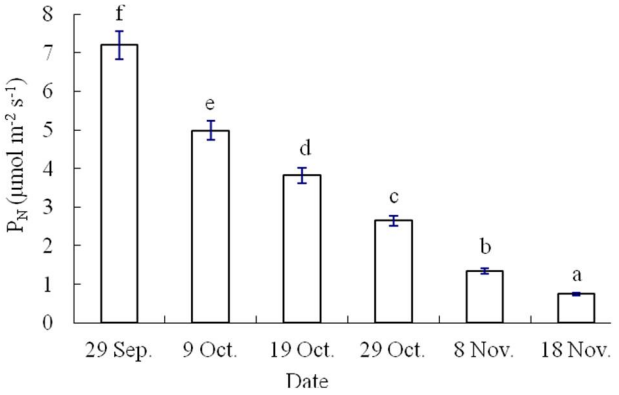

Fig. 3. Changes in the P_N of ginkgo leaves during natural yellowing.

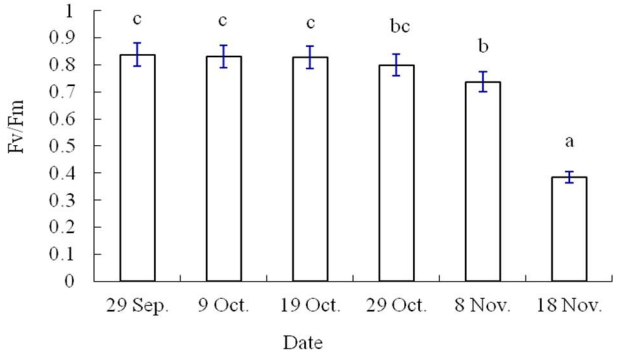

Fig. 4. Changes in the F_v / F_m of ginkgo leaves during natural yellowing.

Figs 3 and 4 showed that the P_N decreased rapidly while the F_v / F_m changed little during leaf natural yellowing, and Figs 5 and 6 showed that the activities of SOD decreased while the content of MDA increased during leaf natural yellowing. The decrease in chlorophyll content significantly reduces photosynthetic efficiency and organic matter synthesis (Afrin *et al.* 2021). In order to maintain normal growth and development, plants have to use stored organic matter to decompose it into soluble sugars and provide necessary energy for the plants. The various organic substances accumulated in yellowing plants, such as soluble sugars and proteins, can increase the concentration of cell sap, reduce its osmotic potential, maintain a certain osmotic pressure, and regulate the water content of plant through osmotic regulation, thereby reducing damage to cells. The production and clearance of reactive oxygen species within cells are usually in a dynamic equilibrium state. When the level of reactive oxygen species is very low, it will not harm the cells. However, when plants are subjected to stress, excessive accumulation of reactive oxygen species disrupts the balance, leading to membrane lipid peroxidation, decreased SOD protective enzyme activity, and the production of more membrane lipid products, thereby damaging membrane integrity (Wang *et al.* 2020). Photosynthetic abilities gradually decreased as the leaves yellowing.

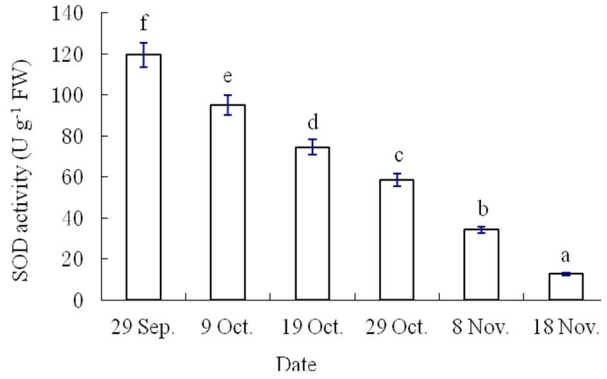

Fig. 5. Changes in the activities of SOD of ginkgo leaves during natural yellowing.

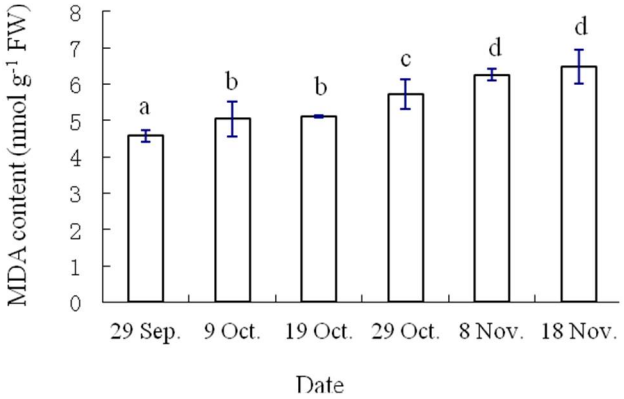

Fig. 6. Changes in the contents of MDA of ginkgo leaves during natural yellowing.

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