BIOLOGIA PLANTARUM 43 (4): 545-548, 2000

# Physiological and biochemical changes during seed filling in relation to leaf senescence in soybean

J. FU*, B. HUANG* and G. ZHANG**

*Department of Horticulture, Forestry and Recreation Resources, Kansas State University, Manhattan, KS 66506, USA**
*Department of Agronomy, Shandong Agricultural University, 271018 Shandong, PR China***

## Abstract

Field experiments with *Glycine max* (L.) Merr. cv. Ludou 11 and Ludou 4 were conducted to evaluate changes in photosynthetic rate, antioxidative enzyme activity, soluble protein, chlorophyll (Chl) and carotenoid (Car) contents in relation to leaf senescence during seed filling period. Photosynthetic rate, soluble protein content, catalase and peroxidase activities were the highest at 25 days after flowering (DAF). Chl *a*, Chl *b* and Car contents reached the maximum at 15 DAF and rapidly decreased after 33 DAF.

*Additional key words*: antioxidative enzyme activity, catalase, carotenoids, chlorophyll, *Glycine max*, peroxidase, photosynthetic rate.

## Introduction

Seed yield of soybean is closely related to the physiological conditions of leaves during the reproductive stage (Hanway and Weber 1971, Egli and Leggett 1973). Sugars allocated to seeds during seed formation and filling mostly come from the photosynthates produced after flowering. Therefore, delaying leaf senescence of leaves after flowering and increasing photosynthesis at seed filling would improve seed dry matter accumulation (Hayati *et al.* 1995).

Leaf senescence is an integral part of plant development. Net photosynthetic rate of leaves often declines prior to the completion of seed growth, being an early event in soybean leaf senescence and representing a limitation to a yield (Boote *et al.* 1978). The factors responsible for the initiation of leaf senescence are unknown. Hurkman (1979) suggested that the first symptoms of aging occurred in chloroplasts. One of the earliest change associated with leaf senescence is the decrease in total soluble protein content and increase in abscisic acid concentration (Martin and Thimann 1972, Peterson *et al.* 1973, Wittenbach 1977, Joyce and Thomas 1980). Catalase (CAT), superoxide dismutase (SOD), and peroxidase (POX) play an important role in clearing away active oxygen. These enzymes can be used as markers of leaf senescence. Drought-induced membrane lipid peroxidation may play an important role in drought-accelerated senescence of soybean leaves (Xu and Zou 1993). Toivonen and Sweeney (1998) reported that SOD and POX were higher in broccoli cultivar with a stable Chl content than the cultivar with a declined Chl content, but CAT is not important in providing resistance to Chl loss in broccoli. Generally, SOD, POX, and CAT activities decline, and malondialdehyde (MDA) content increases with increasing age of leaves (Guo *et al.* 1998, Li *et al.* 1998). But, it has been also reported that SOD activity increased firstly, and then decreased with advancing leaf age (Wu 1993). The rate of senescence is influenced greatly by environmental conditions (Nooden 1985a,b, Phillips 1984, Pierce 1984).

The objective of study was to determine the changes in photosynthesis, activities of antioxidant enzymes, soluble protein, chlorophyll and carotene contents during seed filling in two cultivars of soybean differing in seed filling period length and seed yield.

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*Received* 16 November 1999, *accepted* 15 April 2000.
*Abbreviations*: Car - carotenoids; CAT - catalase; Chl - chlorophyll; DAF - days after flowering; MDA - malondialdehyde; $P_N$ - net photosynthetic rate; POX - peroxidase.
Fax: (+86) 532 6949, e-mail: jfu@oz.oznet.ksu.edu

## Materials and methods

The experiments were conducted in research farm of the Shandong Agricultural University, China in 1997 and 1998. Two soybean [*Glycine max* (L.) Merr.] cultivars Ludou 11 and Ludou 4 were planted on June 16, 1997 and on June 1, 1998. Plants were sufficienly supplied with urea and nitrogen.

The youngest fully expanded leaves were selected from 20 plants and were tagged with paper labels at the initiation of flowering (R1) on July 28, 1997. Labeled leaves from three plants were sampled at 0, 8, 15, 33 and 41 days after flowering (DAF). Leaves were placed quickly in liquid nitrogen and stored at -40 °C. To determine soluble protein content, and CAT and POX activities, 1 g of frozen leaves was homogenized with 5 $cm^3$ of 50 mM buffer solution (pH 7.0) containing 0.07 % of $NaH_2PO_4 . 2 H_2O$ and 1.6 % $Na_2HPO_4 . 12 H_2O$, grounded with a mortar and pestle, and centrifuged at 15 000 *g* for 25 min in a refrigerated centrifuge (*Anting Scientific Instrument Company*, Shanghai, P.R. China). Soluble protein content was measured using the method of Bradford (1976). Activities of CAT and POX were measured using the method of Chance and Maehly (1955). Chlorophyll (Chl) and carotenoid (Car) contents were determined in 80 % aceton extract according to the method of Arnon (1949). Leaf net photosynthetic rate was measured at 0, 8, 21, 29, 41 DAF in 1998 using portable photosynthetic system *LI-6200* (*Licor*, Lincoln, USA).

The experiments were done incompletely randomized design and all measurements were made on multiple leaves from three plants. Data were analyzed using the general linear model of the *Statistical Analysis System*.

## Results

For both cultivars, net photosynthetic rate ($P_N$) increased from 0 to 8 DAF, then maintained steadily high until 29 DAF; thereafter, declined sharply (Fig. 1*A*). Significant difference in $P_N$ between Ludou 11 and Ludou 4 was found only at 41 DAF, with the former having higher $P_N$.

Soluble protein content of both cultivars increased from 0 to 8 DAF and maintained steadily high until 25 DAF. Then, it decreased from 25 to 33 DAF, especially in Ludou 4. But, thereafter, soluble protein content increased dramatically for both cultivars (Fig. 1*B*). No significant difference in soluble protein contents was observed between both cultivars, except at 41 DAF.

Newly expanded leaves of both cultivars had low CAT activity (Fig. 1*C*). CAT activity increased form 0 to 8 DAF and reached to the maximum at 25 DAF, then declined rapidly reaching at 41 DAF the similar level as at 0 DAF for Ludou 11 and at 33 DAF for Ludou 4. Leaves of Ludou 11 had mostly higher CAT activity than leaves of Ludou 4, except at 0 and 41 DAF.

Newly expanded leaves had the mimimum POX activity in both cultivars. The POX activity increased from 8 DAF to the maximum at 25 DAF for Ludou 4 and at 33 DAF for Ludou 11 (Fig. 1*D*). Significantly lower POX activity was found in Ludou 4 than in Ludou 4 at 0, 25, 33 and 41 DAF, but no significant differences at 8 and 15 DAF.

Chl and Car contents were also the lowest in newly expanded leaves of both cultivars. They increased to the highest level at 15 DAF and then rapidly declined thereafter. Significantly higher Chl *a*, Chl *b*, and Car contents in Ludou 11 than in Ludou 4 were observed at 25 DAF. At 15 DAF, only the differences in Chl *b* and Chl *a+b* between Ludou 11 and Ludou 4 were significant.

Table 1. Changes in chlorophyll and carotenoid contents [mg $g^{-1}$(f.m.)] in soybean leaves. Means with the same letter are not significantly different ($n$ = 6).

| DAF | Cultivar | Chl *a* | Chl *b* | Car |
|---|---|---|---|---|
| 0 | Ludou 11 | 1.419 a | 0.391 a | 0.460 a |
| | Ludou 4 | 1.326 a | 0.308 a | 0.463 a |
| 8 | Ludou 11 | 1.792 a | 0.451 a | 0.630 a |
| | Ludou 4 | 1.567 a | 0.519 a | 0.481 a |
| 15 | Ludou 11 | 2.002 a | 0.589 a | 0.713 a |
| | Ludou 4 | 1.982 a | 0.552 b | 0.653 a |
| 25 | Ludou 11 | 1.509 a | 0.503 a | 0.480 a |
| | Ludou 4 | 0.834 b | 0.268 b | 0.673 b |
| 33 | Ludou 11 | 0.982 a | 0.292 a | 0.356 a |
| | Ludou 4 | 0.749 a | 0.201 a | 0.277 a |
| 41 | Ludou 11 | 0.791 a | 0.222 a | 0.289 a |
| | Ludou 4 | 0.733 a | 0.237 a | 0.259 a |

## Discussion

Delaying leaf senescence is very important during seed maturation and grain yield (Camp *et al.* 1982). Our results showed that the youngest leaves at flowering stage needed 8 d to be fully expanded and to reach high photosynthetic rate. Then, they maintained high photosynthetic rate for 17 - 21 d. This period could be named effective photosynthetic period. Thereafter, leaf senescence and decrease in $P_N$ occurred. Retarding leaf senescence by fertilizing and watering could increase the duration of effective photosynthetic period.

Changes in soluble protein contents were similar to that of the photosynthetic rate. But, when leaves were dying, soluble protein content increased dramatically for both cultivars (Fig. 1*B*). This might be attributed to dissolution of insoluble proteins into soluble proteins. The decrease in Chl and Car contents usually occurred during senescence (*e.g.* Murchie 1999). Our results also confirm decreases in Chl and Car contents during the senescence of soybean leaves.

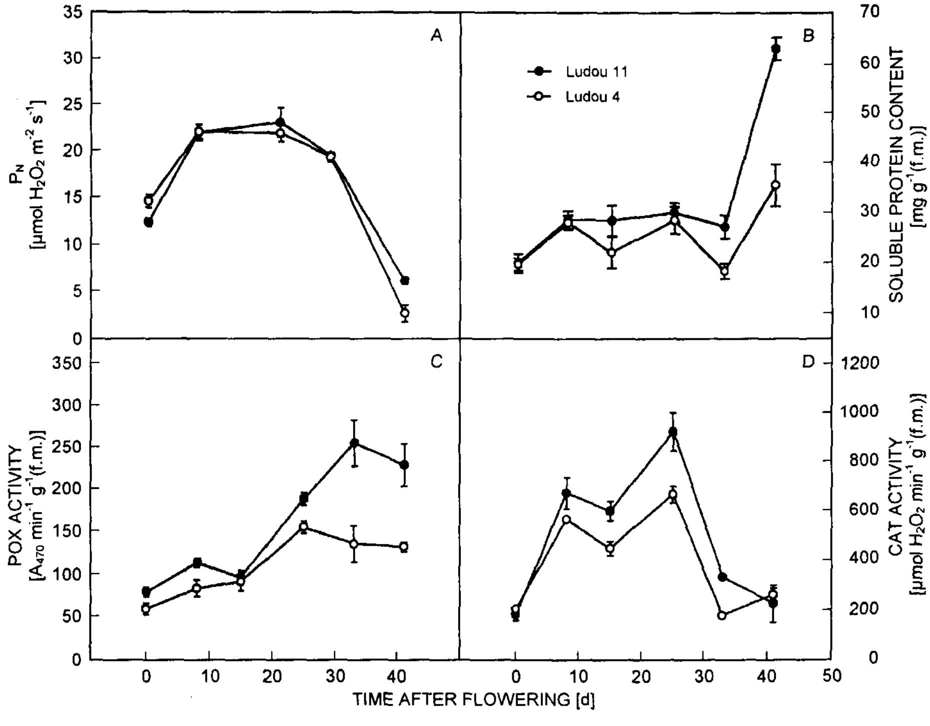


Fig. 1. Net photosynthetic rate (*A*), soluble protein content (*B*), peroxidase activity (*C*), and catalase activity (*D*) in response to soybean leaf age.

The senescence induces oxidative stress resulted from the production of active oxygen species (Guo *et al.* 1998, Li *et al.* 1998). Under optimum growth conditions, plants maintain a balance between producing and scavenging of active oxygen species. But with leaf senescence, the balance is often disturbed either by increasing the production of active oxygen species or by decreasing the scavenging ability in the cells (Bowler *et al.* 1992). Observed increase in POX activity after 8 DAF suggested that leaves had increasing ability to catalyze hydrogen peroxide-dependent oxidation of substrate. But, decrease in POX after 25 DAF for Ludou 4 and 33 DAF for Ludou 11 shown that this ability declined with leaf senescence. Higher CAT activity from 8 to 25 DAF showed higher ability of leaves to break down $H_2O_2$. The decrease in CAT activity after 25 DAF would result in the accumulation $H_2O_2$, which can react with $O^{\cdot}_2$ to produce hydroxyl radicals via the Herbert-Weiss reaction (Elstner 1982, Bowler *et al.* 1992). These radicals can induce lipid peroxidation in the cell.

There was a slight difference in leaf senescence progress between the two cultivars. Ludou 11 with longer duration of seed filling period and higher seed yield, had higher soluble protein content, CAT activity, POX activity, Chl content and carotenoid content during senescence than Ludou 4.

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