# Effect of photoperiod length on leaf ontogenesis in spinach plants (Spinacia oleracea var Nobel) as related to photosynthetic O2 Produktion : RuBisCo capacity and total sugar content

Autor(en): Frosch, Silvia / Tsala, Guy / Degli Agosti, Robert

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# EFFECT OF PHOTOPERIOD LENGTH ON LEAF ONTOGENESIS IN SPINACH PLANTS (SPINACIA OLERACEA VAR NOBEL) AS RELATED TO PHOTOSYNTHETIC O<sub>2</sub> PRODUCTION, RUBISCO CAPACITY AND TOTAL SUGAR CONTENT

BY

# Silvia FROSCH, Guy TSALA, Robert DEGLI AGOSTI and Hubert GREPPIN<sup>+</sup>

Keywords: Photosynthetic O<sub>2</sub> production, Rubisco, total soluble sugar content, photoperiodism, ontogenesis, flowering, *Spinacia oleracea*, long day plants.

Abbreviations: Chl chlorophyll LDs long days

LDPs long day plants Rubisco Ribulose-1,5-bisphosphate carboxylase SDs short days SDPs short day plants

#### ABSTRACT

The acclimation to increased photoperiod length was studied in vegetative and floral spinach plants with respect to changes in photosynthetic activity, ontogenesis and dry weight yield.

Heterogeneity in size and physiological activity between the primary and secondary leaves was found under short day as well as upon transfer to continuous light. The secondary leaves emerge in SD conditions at a time when the primary leaves are about half maximal size with about 50% of the final chloroplast components. Rapid enlargement of the secondary leaves is accompanied by a second rise in photosynthetic  $O_2$  production. Transfer of young plants primarily stimulates metabolic activity and growth of the primary

<sup>&</sup>lt;sup>1</sup> Physiologie végétale, 3, place de l'Université, CH-1211 Genève 4, Switzerland.

leaves. However, plants whose first leaves were close to maturity upon transfer predominately increase development of secondary leaves. The physiological activities of leaves are higher in continuous light but the life span of the leaves is considerably shorter.

Photosynthate production is limited in SD conditions, which in turn limites synthesis of chloroplast constituents (especially Rubisco capacity) and finally restricts leaf growth. Prolongation of the daily incident radiation leads to increases in chloroplast components, growth and dry weight yield. The extent of acclimation is higher for Rubisco capacity, soluble protein, total soluble sugar content and dry weight compared to chlorophyll content,  $O_2$  evolution and leaf area expansion.

Rapid modifications are observed once the critical photoperiod is overcome. These changes might however be concomitant with flowering without necessarily directly promoting floral induction.

#### RÉSUMÉ

L'effet de photopériodes inductrices ou non de la floraison est examiné chez les feuilles d'épinard en relation avec l'activité photosynthétique, leur développement et le poids sec.

On observe une hétérogénéité dans la taille et l'activité physiologique entre les feuilles primaires et secondaires aussi bien en jour court qu'en lumière continue. En jour court, les feuilles secondaires apparaissent lorsque les feuilles primaires ont atteint la moitié de leur dimension finale. La croissance rapide des feuilles secondaires s'accompagne d'une augmentation transitoire de la production d'oxygène photosynthétique des feuilles primaires.

Lors du transfert de jeunes plantes en lumière continue on observe d'abord une augmentation de la croissance et de l'activité chez les feuilles primaires. En revanche, pour des plantes plus âgées, le transfert accélère préférentiellement le développement des feuilles secondaires. En lumière continue les activités physiologiques sont plus intenses, mais la durée de vie des feuilles est beaucoup plus courte.

La production photosynthétique est limitée en jour court, ce qui a pour effet de conditionner la synthèse des composants des chloroplates (en particulier la ribulose-bisphosphate carboxylase) et donc finalement de limiter la croissance de la feuille. Une photopériode accrue augmente les composants chloroplastiques donc la croissance et le poids sec. Cette augmentation est plus prononcée pour l'activité de la ribulose-bisphosphate carboxylase, les protéines solubles totales, les sucres solubles totaux et le poids sec, lorsqu'on les compare aux teneurs de chlorophylle, au dégagement d'oxygène et à l'augmentation de la surface foliaire.

Des modifications rapides des sucres totaux solubles et du dégagement d'oxygène photosynthétique sont observées dès que la durée de la photopériode inductrice de la floraison est atteinte.

#### INTRODUCTION

During leaf ontogeny, morphology, size and physiological activities vary considerably. Changes in various photosynthetic parameters have been studied in a number of different plants [for reviews see 19, 22, 26, 27]. External factors, such as light or temperature, may greatly influence the rapidity and extent of leaf development. Balanced changes in many leaf components during photosynthetic acclimation to different light levels are necessary to insure that a single reaction does not limit lightsaturated rates of photosynthesis under natural conditions [3, 25]. Since the leaf is the site of daylength perception, changes in chloroplast function, related to the initiation of flowering, has been followed in spinach (Spinacia oleracea var. Nobel). The increase in daylength, once the critical photoperiod is overcome (11-12 h light), results in floral induction [14]. The induced state of the leaves was characterized by differences in the ultrastructure of the chloroplasts [6, 7]. During induction the leaves produce "signals" that are transmitted to the apex. The first events of flower evocation in the apical meristem are observed after about 15 h of continuous light [1]. Photoperiodic induction in spinach plants is thus a very rapid process.

This paper examines some elementary parameters of chloroplast function during the ontogeny of primary and secondary leaves of spinach under photoperiodic conditions that induce flowering. Because of its potential rate-limiting role in photosynthesis and respiration [10] Rubisco was chosen as marker enzyme for the stroma. Chlorophyll content was used as marker for thylakoid changes. Measurements of photosynthetic activity were based on O<sub>2</sub> production determined polarographically in whole leaves. Since the production and circulation of free sugars are often considered to be involved in floral induction, at least in LDPs [2, 5], total free sugar content was determined colorimetrically as an indication of photosynthetic product accumulation capacity. Soluble protein content represents a general indicator for changes in cellular metabolism whereas dry weight accumulation and leaf area expansion represent productivity (yield) and growth respectively.

We show that chloroplast constituents and metabolism acclimate to the prolonged daily incident radiation and that the timing and the extent of the acclimation varies for the different parameters studied.

### MATERIAL AND METHODS

#### Plant Material and Growth Conditions

Achenes of spinach (*Spinacea oleracea*, var. Nobel) were obtained from selected plants of the experimental botanical station in Geneva, Switzerland [13]. Plants were grown at 20° C for 2 to 6 weeks (SDs: 8 h fluorescent white light at 20.4 W m<sup>-2</sup>-16 h darkness). Illumination was from fluorescent lamps (Sylvania, TL33, daylight, 40 W). Plants were transferred 16, 21 or 28 days after sowing to continuous light or allowed to remain in SD conditions.

#### Leaf sampling, extraction procedures and test methods

For growth studies, leaves were harvested in the middle of the SD light period and simultaneously from plants that had been transferred to continuous light. Leaves of 4 plants per treatment were photocopied and the area was evaluated, planimetrically.

They were then dried at 70° C for dry weight measurement. Leaves of 4 different plants were suspended in 80% acetone, kept in darkness at 0° C for 24 h and Chl was determined according to MacKinney 1941 [15]. An additional 4 pairs of leaves were homogenized and Rubisco activity ( $\mu$  moles NaHCO<sub>3</sub>.min<sup>-1</sup>) and soluble protein content were determined as described earlier [12]. Photosynthetic O<sub>2</sub> production was measured in individual leaves polarographically [23]. Sugars were extracted by incubating leaves or petioles in hot 80% ethanol for 30 min. The extracts were partially purified with a SEP PAK C18 column (Waters Associates, Inc.) and determined using the anthrone method [28]. The extract was incubated with anthrone solution (0,2% w/v H<sub>2</sub>SO<sub>4</sub>) at 90° C for 10 min. The green product was measured at 630 nm (Unicam SP 1700 spectrophotometer). Glucose equivalents were calculated from a standard curve obtained with pure analytical grade glucose.



Fig. 1.

Time course of changes in leaf area and dry matter in the primary and secondary leaves of spinach plants grown in SDs (o—o) and upon transfer to continuous light. ↓ represents time of transfer to continuous light, either after 16 SDs (•), 21 SDs (♥) or 28 SDs (♡). The coefficient of variation from two experiments with four replicates (each with 4 pairs of leaves) is 6-8% for leaf area and 4-6% for dry weight.



FIG. 2.

Time course of changes in Chl content, Rubisco capacity and soluble protein accumulation in the primary and secondary leaves of spinach plants grown in SDs (o—o) and upon transfer to continuous light. ↓ represents time of transfer to continuous light, either after 16 SDs (•), 21 SDs (▼) or 28 SDs (▽). The coefficient of variation from two experiments with four replicates (each with 4 pairs of leaves) is 2-8% for Rubisco and soluble protein and 5-10% for Chl.



FIG. 3.

Time course of Chl content, Rubisco capacity, soluble protein content and dry matter level on a leaf area basis in primary and secondary leaves of spinach plants grown in SDs (o—o) and upon transfer to continuous light.  $\downarrow$  represents time of transfer to continuous light either after 21 SDs ( $\nabla$ ) or 28 SDs ( $\nabla$ ).

#### RESULTS

Leaf area expansion and dry matter accumulation in plants growing under SDs and after transfer to continuous light are shown in Fig. 1. The size of the primary leaves increases gradually in plants grown under an 8 h photoperiod. Leaves reach their final size about 35 d after sowing (Fig. 1a). In SDs primary leaves accumulate dry weight slowly for an even longer growth period (Fig. 1b). The secondary leaves appear 21-24 days after sowing, their area expands more rapidly and the rate of dry matter yield is also increased (Fig. 1d) in comparison to the primary leaves (Fig. 1c).



Fig. 4.

Time course of O₂ production in the primary leaves of spinach plants grown in SDs (o—o) and upon transfer to continuous light. ↓ represents time of transfer either after 14 SDs (•) or 22 SDs (♥). The coefficient of variation from two experiments with four replicates (each with 4 pairs of leaves) is 6-10%.

Upon transfer to continuous light (16, 21 or 28 days after sowing), the primary leaves enlarge more rapidly when compared to leaves growing under SDs (Fig. 1a). Dry weight also accumulates at a rate which exceeds that observed in SD leaves (Fig. 1b). The younger the leaf at the time of transfer to continuous light the greater is the increase of dry weight in the primary leaves. Transfer of plants to continuous light also greatly accelerates the appearance of the secondary leaves which become several times larger and exhibit much higher dry matter yield than that recorded in SD plants (Fig. 1c, 1d).

Physiological parameters such as Chl content, Rubisco capacity and soluble protein content of leaves grown under SD conditions remain several fold lower as compared to the levels reached after transfer to continuous light (Fig. 2). Transfer of young plants predominately stimulates accumulation in the primary leaves (Fig. 2a, 2b, 2c) whereas older plants predominately increase metabolic activity of the secondary leaves upon transfer (Fig. 2d, 2e, 2f). Thus, transfer to a floral inductive continuous light regime causes an increase in all measured physiological parameters but the parallel relationship with leaf area and dry weight is basically maintained while the extent of these changes is dependent upon leaf age and leaf insertion level. An earlier onset of senescence phenomena also occurs upon transfer when compared to the SD controls (Fig. 2).





Time course of  $O_2$  production and Rubisco capacity in primary leaves of spinach plants grown in SDs (0-0) and upon transfer to continuous light ( $\nabla$ --- $\nabla$ ).

When the measured parameters are plotted as a function of leaf area the transfer to continuous light causes rapid increases in Chl, Rubisco, protein and dry matter accumulation (Fig. 3). All responses reach their maximum 4-5 days after transfer. Maximal amounts are 1.5 to 2.5 fold higher than in primary leaves of the SD control. Chl content and Rubisco capacity increase considerably already during the first 24 h after the transfer of 21 d SDPs whereas highest accumulation rates of soluble protein content and dry matter are observed between day 1 and 3 after the transfer (Fig. 3a-d). Emerging secondary leaves accumulate 3-5 times more Chl, Rubisco, soluble protein and dry weight upon transfer (Fig. 3e-h).





Time course of total soluble sugar content in the primary leaves and their petioles of spinach plants grown in SDs (—) or upon transfer to continuous light (---). Organs from 16 plants were used per extract and results presented are the arithmetic mean of 3 extracts.

The increased amount of all parameters are indicative of higher metabolic activity of the chloroplasts upon transfer which may result in higher organelle content per cell and/or increases in leaf thickness. Decreases of physiological activities on a leaf area basis might reflect increases in cell volume and vacuole size.

When Chl, Rubisco and soluble protein content were calculated on a dry weight basis (as a parameter of leaf productivity, values not presented) transitory increases of the ratios were found especially after transfer of young plants. However, 2 to 3 days after transfer the ratios either are equal to the SD ratios or drop below the SD levels.

Photosynthetic  $O_2$  production in the primary leaves reaches a peak after 21 SDs and then a second peak after 28 SDs (Fig. 4). The second peak coincides with the emergence of the secondary leaves (compare with Fig. 1). In plants, grown from time of sowing under continuous light however, oxygen production exhibits a single and very high peak 13 days after sowing (results not presented). Thus reduction of the

light quantity in SDs not only slows the development of the leaves but also changes the ontogenetic pattern of oxygen production. Upon transfer of 14 SD old plants to continuous light,  $O_2$  production increases rapidly over the SD control. However, this is not the case when older plants (22 SDs) are transferred where  $O_2$  production rapidly falls below the SD levels (Fig. 4a). Expressing  $O_2$  production on a Chl basis (Fig. 4b) shows that the two peak pattern in SD leaves remains. However transfer to continuous light causes a rapid decrease in photosynthetic  $O_2$  production under the SD controls.

The parameters were measured over much shorter time intervals (1-4 h) following prolongation of the daylength (Fig. 5). During the first 4 hours after the transfer to continuous light (the critical photoperiod length is 11-12 h)  $O_2$  production is increased both on a leaf and on a Chl basis when compared to the SD control (Fig. 5a, 5b). Rubisco capacity on the other hand increases relatively slowly over the SD control, indicative of preferential Rubisco synthesis as compared to total protein or Chl synthesis.

In vitro determinations of enzyme activities are however always substrate saturated. Thus the actual utilization of substrate *in vivo* under different environmental conditions remains unknown. By determining products, e.g. total soluble sugar content of leaves, it is evident that shortly after transfer to continuous light sugar content doubles (Fig. 6). During the dark period of the SD cycle soluble sugar content rapidly drops to very low levels. In petioles the same pattern is observed but the difference between the treatments is less pronounced and during darkness total soluble sugar content remains relatively elevated.

#### DISCUSSION

Regulation of photosynthesis and its influence on growth, productivity and reproduction accompanied by changing source-sink relationship due to unfolding of further leaves is a complex topic. During the acclimation to photoperiod length in vegetative and floral spinach plants, development of photosynthetic parameters are increased along with leaf area and dry matter. Growth, developemnt and dry weight accumulation under SDs in the primary leaves is slow and depends strongly on the photosynthetic export capacities of the cotyledons. At half maximal size of the primary leaves a second pair emerges and its expansion rate doubles (rate calculations not presented). Heterogeneity in size between primary and secondary leaves is observed in SD plants and upon transfer to continuous light, as well as in plants grown in continuous light from sowing. Increase in photoperiod length accelerates the rate of emergence of the following leaves while heterogeneity remains. Thus light acclimation only modulates the timing and extent of the developmental pattern and leaf heterogeneity is apparently genetically fixed for this spinach variety. An alternating pattern of leaf metabolism is observed under SD conditions i.e. predominately anabolic during day and catabolic during night [8].

Photosynthetic  $O_2$  production is highest at the beginning of the night period and total free sugar content varies diurnally. Soluble sugar content drops to very low values in leaves whereas the diurnal variation is less pronounced in petioles due to its dependence on export rates from the leaves. Starch content varies diurnally and there are diurnal changes in dry weight of leaf pieces [8]. Rubisco capacity, however, is very stable in spinach although diurnal variations of Rubisco activity have been observed in soybean leaves [24]. In contrast to the ontogenetic evolution of Chl content and Rubisco capacity, photosynthetic  $O_2$  production in SD leaves shows a double peak pattern. The second peak coincides with the emergence of the secondary leaves. The early decrease in  $O_2$  production after 20 SDs may thus be an indication of a lack of a sink. Photosynthetic  $CO_2$  fixation increases when sinks (leaves, flowers, pods) are established [11, 16, 21].

Extension of the photoperiod leads to a large increase in chloroplast parameters as well as in growth and yield. Chl and Rubisco accumulation precede increases in soluble protein and dry weight following transfer of young plants (21 SDs) to continuous light. Photosynthetic  $O_2$  production increases in 14 SD old plants upon transfer whereas in older plants (22 SDs)  $O_2$  evolution increases only transitorily during 4 h and then declines. The extent of acclimation of 21 SD old plants upon transfer is higher for Rubisco capacity, soluble protein content, dry weight and total sugars when compared to Chl content and  $O_2$  evolution. Thus calvin cycle constituents and activity may acclimate more than electron transport [17]. The increases during continuous light might not be constant with time. Accumulation of substrates may vary rhythmically as indicated by decreases in  $O_2$  production and total soluble sugar content during the extended light period.

The acclimation potential in first leaves is greatest in early blade expansion and then decreases with maturity. The life span of the primary leaves is considerably shortened and senescence is observed much earlier when compared to SD treatments. The earliest indication of senescence may involve mitochondrial activity [8]. Electron transport capacity might also be prone to early aging as indicated by decreased  $O_2$ production after transfer of 22 SD old plants. Photosynthesis, photo and dark respiration decreased more rapidly in spinach plants adapted to 12: 12 h light-dark periods than plants adapted to 7: 17 h light-dark cycles [17].

While the dependence of some chloroplast functions on photoperiod length could be established, their role in the process leading to flowering remains unclear. LPDs have a requirement for a so called "high intensity light period" in addition to control by phytochrome [5, 9, 18]. Long term irradiations lead to changes in leaf growth and net  $CO_2$  uptake and/or output [18]. The rapid modification of  $O_2$  production and sugar content correlate in time with the transition to flowering. These changes might however only be concommitant with floral induction without constituting part of the "signals" transmitted to the apex. The extent of the acclimation responses of Chl, Rubisco and soluble protein content during continuous irradiation rather point to a major contribution to flower development than to a primary role in the production of the "signals".

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