# COMPARISON OF PHOTOSYNTHETIC PERFORMANCES IN TWO GENOTYPES OF BEAN DEEPOXIDATING VIOLAXANTHIN QUICKLY AS WELL AS SLOWLY

# L. TÉCSI, K. MARGÓCZI, E. TAKÁCS AND I. MARÓTI

Research Group of Hungarian Academy of Sciences at Botanical Department of József Attila University H-6726 Szeged, Lövölde u. 42. Hungary

(Received: January 5, 1989)

#### Abstract

Differences were studied in the performance (the dry matter production in connection with CO2 assimilation, leaf area, ratio of photosynthetizing tissues, malate, sucrose, starch contents of the leaf) between the XQ and XS genotypes (deepoxidating violaxanthin quickly (XQ) as well as slowly (XS) during the induction period of photosynthesis) of the C3 type bean. We observed the differences of the acclimation of the plants were grown at 3 light regimes: medium light (ML): 200  $\mu$ mol.m<sup>-2</sup>.s<sup>-1</sup> photosynthetic photon flux density (PPFD) and 16 h — 8 h light dark period (LDP); low light (LL): 100  $\mu$ mol.m<sup>-2</sup>.s<sup>-1</sup> PPFD and 16 h — 8 h LDP; short light (SL): 200  $\mu$ mol.m<sup>-2</sup>.s<sup>-1</sup> PPFD and 30 min — 15 min LDP.

Dry matter reducing effect of the LL and the SL treatments seemed to be in connection with the low CO2 assimilation rate measured at the PPFD where the plants were grown and with the low quantum yield. Within a genotype the higher sucrose and the lower starch proportion on the effect of light treatments may be in connection with the higher shoot and lower root ratios. Comparing the XQ and XS genotypes of bean, we found that the malate, sucrose levels are lower and the starch level is higher in the leaves of the XQ than those of the XS genotypes.

The quantum yield may be in positive correlation with chlorophyll a + b content and negative correlation with the light compensation point.

Key words: genotype, CO2 assimilation, biomass, violaxanthin, sucrose, starch, malate

## Introduction

The differences in the performance of genotypes may be in close connection with the photosynthetic pathways. For instance such differences are shown by the variation found between the photosynthetic pathways of the C3 and C4 species of *Panicum* genus (DOWNTON, 1975) and between the genotypes of *Zea mays* deepoxidating violaxanthin quickly (XQ) as well as slowly (XS) during the induction period of photosynthesis (MARÓTI, 1986). Our aim was to study the differences in the performance (the dry matter production being in connection with  $CO_2$  assimilation, leaf area, ratio of photosynthetizing tissues, malate, sucrose, starch content of the leaf) between the XQ and the XS genotypes of the C3 type bean. We observed the differences in the acclimation of the plants grown at medium and low photosynthetic photon flux densities and short light dark period.

The carboxylating step is well characterized by the slope of the initial part of the curve of CO<sub>2</sub> assimilation rate versus incident photosynthetic photon flux density. This initial slope of the curve is the incident quantum yield that is lower in the C3 plants (0.052 mol CO2.mol-1 photon) than in C4-NADP-ME plants (0.065 mol CO<sub>2</sub>.mol<sup>-1</sup> photon) (EHLERINGER and PEARCY, 1983). Since the optimal electron transport needs equilibrium between production and consumption of the reduction power (NADPH) in the chloroplast, therefore the export of the reduction power in the form of malate plays an important role in the acclimation to the changing conditions (SCHEIBE et al. 1986). On the one hand, the photosynthetic malate is the precursor of the tricarboxylic acid cycle in the mitochondrium, because the amino acids of the tricarboxylic acid cycle are synthetized from the photosynthetic CO2 fixation (KENT, 1979). On the other hand, the malate exported from the chloroplast carries the reduction power to the peroxisomes (TOLBERT, 1979), and to the cytoplasm. The increase of the CO<sub>2</sub> concentration rises the malate level and reduces the nitrate level since the malate oxidation is the main NADH source for the cytoplasmatic nitrate reduction (NEYRA and HAGMAN, 1976; MARIGO et al., 1985). Certain plants can store malate in inverse quantity as nitrate in their vacuoles (GERHARDT AND HELDT, 1984; MARIGO et al., 1985).

The rate of the sucrose synthesis is in close correlation with the rate of  $CO_2$  assimilation through the fructose-2,6-bisphosphate regulation system (STITT et al., 1987). The vacuoles of the mesophyllum cells are temporary pools of sucrose in light if sucrose productivity surpasses the uptake capacity of phloem (KAISER and HEBER, 1984). The excess sucrose synthesis gives a signal for the fructose-2,6-bisphosphate regulating system to start the starch synthesis (PREISS, 1986).

The starch content of the leaves is in inverse ratio to the daily photosynthetic period (CHATTERTON and SILVIUS, 1979).

Genotype pairs was found in some plant species (maize, bean, sunflower) on the basis of the leaf anatomy, chloroplast ultrastructure and physiological light acclimation. The rate of the deepoxidation of violaxanthin (i. e. the rate of developing of protongradient in thylakoids) quicker, the rate of quenching of chlorophyll-a fluorescence in the M—T period (i. e. the start of the non-cyclic electrontransport) and the rate of oxygen evolution is slower in the XQ genotypes in the XS genotypes (PATAKY and MARÓTI, 1985; WALKER, 1985; MARÓTI, 1986) during the induction period of photosynthesis. The XQ genotypes have lower dry matter, leaf thickness and growth rate, higher water content than the XS genotypes have (MARÓTI and MARGÓCZI, 1984; MARGÓCZI and MARÓTI, 1985). The quantity of appressed membrance in the chloroplasts of the XQ genotypes is greater, the number of grana, the sizes of the loculi are less than in the XS genotypes (PATAKY and MARÓTI, 1985; MARÓTI, 1986).

#### Materials and Methods

The comparison of the photosynthetic performances was carried out on the XQ and the XS genotypes of bean (*Phaseolus vulgaris* L. 'Cherokee').

The bean plants were grown for 40 days in 600 cm<sup>3</sup> plastic pots in the mixture of sand-perlit (I:I) in phytotron with 330  $\mu$ mol.mol<sup>-1</sup>, CO<sub>2</sub> content 8.4  $\mu$ mol.mol<sup>-1</sup>, saturation deficit of water vapour in the air, and with a temperature of 23 C.

The three light treatments were: medium light (ML): 200  $\mu$ mol.m<sup>-2</sup>.s<sup>-1</sup> photosynthetic photon flux density (PPFD) and 16 h — 8 h light dark period (LDP); low light (LL): 100  $\mu$ mol.m<sup>-2</sup>.s<sup>-1</sup> PPFD and 16 h — 8 h LDP; short light dark period (SL): 200  $\mu$ mol.m<sup>-2</sup>.s<sup>-1</sup> PPFD and 30 min — 15 min LDP. The light sources were fluorescent tubes (Tungsram F 33 types).

The ML and the LL plants were kept in darkness for 8 hours and the SL plants were kept in darkness for 30 minutes then their developed 1. trifoliate leaves were cut off. The isolated leaves were illuminated with 800  $\mu$ mol.m<sup>-2</sup>.s<sup>-1</sup> PPFD light in humid 26 C air containing 340  $\mu$ mol.mol CO2 for 30 minutes, then they were fixed in liquid air and lyophilized. The malate (HOHORST, 1970), the sucrose and the starch content (HANDEL, 1968; DUBOIS et al., 1956) were determined. We measured the change of the rate of CO<sub>2</sub> assimilation with infrared gas analyser (VEB Junkalor: Infralyt 5). The calculations (CO<sub>2</sub> assimilation rate, incident quantum yield, light compensation point) were carried out after LONG and HALLGREEN, (1985); JANAC et al. (1971); CAEMMERER and FARQUHAR, (1981).

From the leaves we took samples for lyophilization, we made chlorophyll analysis (FRENCH, 1960) and determined the ratio of the photosynthetic tissues after fixing with glutaraldehyde (KARNOVSKY, 1965), imbedding in paraffin, making 16 µm thick cross-sections.

#### Results

#### DRY MASS AND THE DRY MASS PROPORTION OF ORGANS (FIGS. 1., 2.)

Dry mass of the total plant, the roots, the stem and the leaves decrease more intensively in the LL plants than in the SL plants comparing to the ML plants. The mass proportion of the roots is lower in the LL and similar in the SL plants compared to the in the ML plants. LL and the SL treatments increase the mass proportion of the stem comparing to the ML.

Dry mass of the total plant and leaves in the XQ is higher than in the XS genotypes at each treatments.

#### LEAF AREA (FIG. 3.)

In the plants grown at the LL and the SL the leaf area is higher than in the plants grown at the ML.

There are no significant differences between the leaf area of the two genotypes.

#### SPECIFIC LEAF MASS (FIG. 3.)

The specific leaf mass of the LL and the SL plants is lower in both genotype. The specific leaf mass of the XQ genotype is slightly higher than that of XS one at the ML.

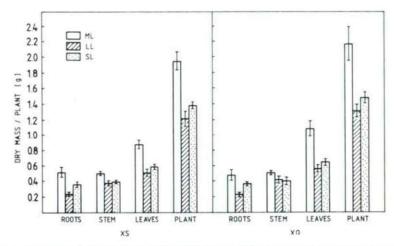


Fig. 1. Dry mass of the organs and the whole plant of the XS as well as the XQ bean genotypes grown at the ML, LL and SL light regimes.

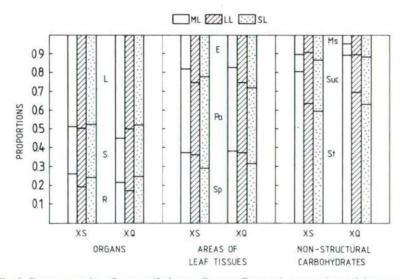


Fig. 2. Dry mass ratios of organs (L: leaves, S: stem, R: roots), proportions of tissue areas in leaf cross-sections (E: epidermis, Pa: palisade parenchyma, Sp: spongy parenchyma) and proportions of the non-structural carbohydrates (MS: monosaccharides, Suc: sucrose, St: starch) in the XS as well as the XQ genotypes of bean grown at the ML, LL and SL light regimes.

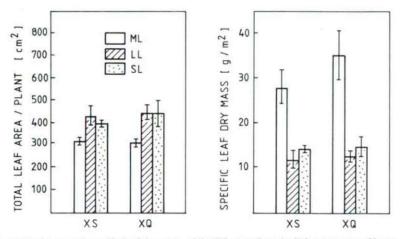


Fig. 3. Leaf area and specific leaf dry mass of the XS as well as the XQ genotypes of bean grown at the ML, LL and SL light regimes.

#### PROPORTION OF TISSUES IN LEAF CROSS-SECTION (FIG. 2., TABLE 1.)

In the LL plants the proportion of palisade, in the SL plants the proportion of spongy parenchymas are reduced comparing to the ML plants. Differences cannot be observed between genotypes with the exception of palisade parenchyma proportion of the SL plants where this proportion is less in the XQ than in the XS genotypes.

 $CO_2$  assimilation rate after 60 minutes of illumination at the PPFD under the plants were grown, incident quantum yield and light compensation point (Fig. 4.)

In the LL and SL plants the  $CO_2$  assimilation rates are lower than in the ML plants. The  $CO_2$  assimilation rate of the XQ genotype is significantly higher than that of the XS genotype with the exception of the SL treatment.

The quantum yield of the LL and the SL plants is lower than that of the ML plants. Advantage of the XQ could be shown only in the LL light regime.

The light compensation point of the SL plants is outstandingly high, but significant genotypical differences could not be observed in either light treatment.

## LEAF TRANSMITTANCE (FIG. 5.)

The leaf transmittance increases slightly in the LL and strongly in the SL plants comparing to the ML plants. There are not any differences between the genotypes.

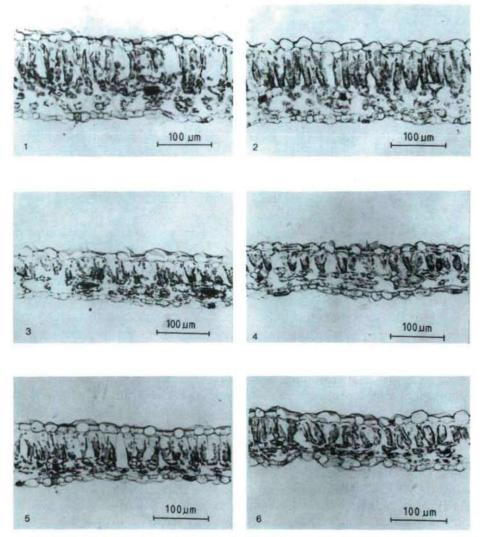


Table 1. Cross-section from 1. trifoliate leaves of the XS and the XQ genotypes of bean grown at the ML, LL and SL light regimes.

1. XS genotype grown at ML 2. XQ genotype grown at ML 3. XS genotype grown at LL 4. XQ genotype grown at LL 5. XS genotype grown at SL 6. XQ genotype grown at SL

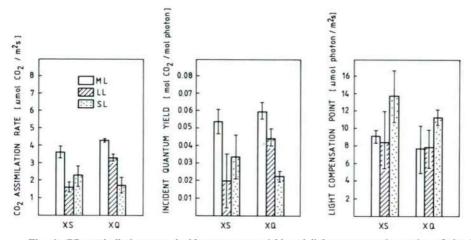


Fig. 4.  $CO_2$  assimilation rate, incident quantum yield and light compensation point of the 1. trifoliate leaves of the XS as well as teh XQ genotypes of bean grown at the ML, LL, and SL light regimes.

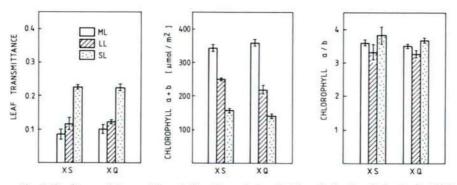


Fig. 5. Leaf transmittance, chlorophyll a+b content and chlorophyll a/b ratio in the 1. trifoliate leaves of the XS as well as the XQ genotypes of bean grown at the ML, LL and SL light regimes.

## QUANTITY OF CHLOROPHYLLS (FIG. 5.)

The chlorophyll a/b decreases in plants grown at the LL and increases in plants grown at the SL comparing to ML. There are not any differences between genotypes. The chlorophyll a + b is lower in the LL and the SL plants than in the ML plants. The chlorophyll a + b is also lower in the XQ than in the XS genotypes except for the plants grown at the ML.

# MALATE, SUCROSE AND STARCH CONTENT OF THE LEAVES IN THE 30TH MINUTE OF THE ILLUMINATION (FIGS. 2., 6.)

The malate level is higher in the leaves of the LL and SL plants than in those of the ML plants. The malate content of the XQ genotype is higher than that of the XS genotype, exept plants grown at the LL light regime.

The sucrose content is higher in the LL and the SL plants than in the ML plants of the XQ genotype, but sucrose content of the XS genotype is similar in each light regimes. The sucrose proportion within the non-structural carbohydrates significantly greater in the LL and the SL plants comparing to the ML plants. The sucrose content of the XQ is lower rhan that of the XS genotype only in the ML plants. The sucrose proportion is also lower in the XQ than in the XS genotype but at each light treatments (Fig. 2.).

Starch level of the leaves is significantly lower in the LL and the SL plants than in the ML plants. The starch content and the starch proportion (Fig. 2.) of the XQ genotype are higher than those of the XS genotype.

#### Discussion

The photosynthetic performance of the plant expresses the efficiency of light conversion to biomass (result of photosynthesis, respiration, photorespiration). The performance could be estimated on the basis of dry matter production in connection with  $CO_2$  assimilation rate, leaf area, ratio of the photosynthetic leaf tissues, and malate sucrose and starch contents of the leaf, respectively.

The stacking degree of the chloroplast membrane system increases in the LL plants (LICHTENTHALER et al., 1981) and decreases in the SL plants (PATAKY and

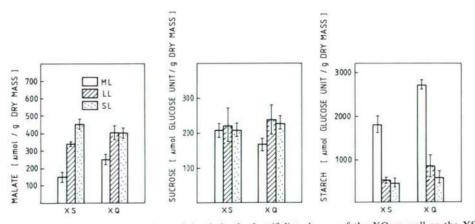


Fig. 6. Malate, sucrose and starch levels in the 1. trifoliate leaves of the XQ as well as the XS genotypes of bean grown at the ML, LL and SL light regimes.

MARÓTI, 1985), but in spite of these facts both light treatments result in a dry mass reduction of bean plants comparing to the ML treatment. The smaller dry matter decrease of the XQ genotype grown at the SL is due to its better light acclimation. The root reducing effect of the LL is consistent with former observations (BJÖRKMAN, 1981) and this is a typical trait of shade acclimation. The LL and the SL treatments result in a phenomenon like shade acclimation: increase of leaf area and reduction of specific leaf mass. The ratios of leaf tissues in cross-section show characteristic differences: in the LL plant the ratio of palisade (cf. LICHTENTHALER et al., 1981), in the SL plants the proportion spongy (cf. MARÓTI and MARGÓCZI, 1984) parenchymas are reduced (Fig. 2.). Dry matter reducing effects of the LL and the SL light regimes may be caused by the reduced CO<sub>2</sub> assimilation rate and quantum yield comparing to the effect of ML light regimes. There is an important difference between the LL and the SL: the light compensation point is unchanged at the LL, but rises at the SL comparing to the ML treatment (Fig. 4.). The latter rise might be due to the weakly developed light harvesting complexes (MARÓTI and TAKÁCS, 1983), which is supported by the small quantity of chlorophyll a + b found in the leaves. The chlorophyll a/b reduction at the LL as well as rise at the SL are consistent with former observations (LICHTENTHALER et al., 1981; MARÓTI, 1982). There may be positive correlation between the incident quantum yield and chlorophyll a+b content as well as negative correlation between the incident quantum yield and light compensation point.

Malate transports reduction power into the cytoplasm for nitrate reduction (MARIGO et al., 1985), into the peroxisomes for glycollate pathway and functions as a carbon source for the anaplerotic operation of the tricarboxylic acid cycle (KENT, 1979). The LL and the SL treatments may reduce the malate level in the cytoplasm which is contrast with other observations (cf. LICHTENTHALER et al., 1981). The rise of sucrose proportion and decline of starch proportion within non-structural carbohydrates in plants grown at the LL and the SL light regimes show the increased transport mobility between chloroplast and cytoplasm in the mesophyll of the leaf of bean plants.

We can establish on metabolite levels of the C3 bean leaves, that the effect of the SL resembles that of the LL treatment.

The lower malate content may be due to the enhanced utilization of malate in the perxisomatic glycollate pathway, in the mitochondrial anaplerotic processes and in the cytoplasmic nitrate reduction. It may be in connection with the higher dry matter of these plants.

The decline of sucrose proportion and the rise of starch proportion within nonstructural carbohydrates in the XQ genotype comparing to the XS genotype (Fig. 2.) show the decreased photosynthate transport from the chloroplasts (HUBER, 1984) and result in an increase in its leaf dry mass (Fig. 1.). Within a genotype the higher sucrose and the lower starch ratio on the effect of light treatments may be in connection with the higher shoot as well as lower root ratios.

Comparing the XQ and XL genotypes of bean with those of maize (TÉCSI, 1987) we found that the malate, sucrose levels are lower and the starch level is higher in the leaves of the XQ than those of the XS genotypes.

### References

- BJÖRKMAN, O. (1981): Responses to different quantum flux densities. In: LANGE, O. L. (ed): Physiological Plant Ecology I. Encyclopaedia of Plant Physiology New Series Vol. 12. A. Pp. 57—101. Springer Verlag, Heidelberg.
- CAEMMERER, S. and FARQUHAR, G. S. (1981): Some relationships between the biochemistry of photosynthesis and gas exchange of leaves. — Planta 153, 376—387.
- CHATTERTON, N. J. and SILVIUS, J. E. (1979): Photosynthate partitioning into starch in soybean leaves. — Plant Physiol. 64, 749—753.
- DOWNTON, W. J. S. (1975): The occurrence of C4 photosynthesis among plants. Photosynthetica 9, 96—105.
- DUBOIS, M., GILLES, K. A., HAMILTON, J. K., REBERS, P. A. and SMITH, F. (1956): Colorimetric method for determination of sugars and related substances. — Anal. Chem. 28, 350—356.
- EHLERINGER, J. and PEARCY, R. (1983): Variation in quantum yield for CO<sub>2</sub> uptake among C3 and C4 plants. — Plant Physiol. 73, 555—559.
- FRENCH, C. S. (1960): Chlorophylls in vivo and in vitro. In: RUHLAND, W. (ed.): Encyclopaedia of Plant Physiology. Vol. 5. Pp. 259—270. Springer Verlag, Heidelberg.
- GERHARDT, R. and HELDT, H. (1984): Measurement of subcellular metabolite levels in leaves by fractionation of freezestopped material in nonaqueous media. — Plant Physiol. 75, 542—547.
- HANDEL, E. (1968): Direct microdetermination of sucrose. Anal. Biochem. 22, 280-283.
- HOHORST, H. J. (1970): L(-)-Malat Bestimmung mit Malat-Dehydrogenase und NAD. In: BERGMEYER, H. U. (ed.): Methoden der Enzymatischen Analyse. Pp. 1544—1548. Akademie Verlag, Berlin.
- HUBER, S. C., RUFTY, T. W. and KERR, P. S. (1984): Effect of photoperiod on partitioning and diurnal rhythms in sucrose phosphate synthase activity in leaves of soybean. — Plant Physiol. 75, 1080—1084.
- JANAC, J., CATSKY, J. and JARVIS, P. G. (1971): Infrared gas analysers and other physical analysers. In: SESTAK, Z., CATSKY, J., Jarvis, P. G. (eds.): Plant Photosynthetic Production. Manual of Methods. Pp. 111—197. Dr W. Junk N. V. Publishers, The Hague.
- KAISER, G. and HEBER, U. (1984): Sucrose transport into vacuoles isolated from barley mesophyll protoplasts. — Planta 161, 562—568.
- KARNOVSKY, M. I. (1965): A formaldehyde glutaraldehyde fixative of high osmolarity for use in EM. Cell Biol. 27, 1371—1381.
- KENT, S. S. (1979): Photosynthesis in the higher plant Vicia faba. V. The role of malate as a precursor of the tricarboxylic acid cycle. — Plant Physiol. 64, 159—161.
- LICHTENTHALER, H. K. (1981): Adaptation of leaves and chloroplasts to high quanta fluence rates. In: AKOYUNOGLOU, G. (ed.): Photosynthesis VI. Photosynthesis and Productivity, Photosynthesis and Environment. Pp. 273—287. Balaban Int. Sci. Service, Philadelphia.
- LONG, S. P. and HALLGREEN, J. E. (1985): Measurement of CO<sub>2</sub> assimilation by plants in the field and the laboratory. — In: COOMBS, J., HALL, S. P., SCURLOCK, J. M. O. (eds.): Techniques in Bioproductivity and Photosynthesis. Pp. 62—94. Pergamon Press, Oxford.
- MARIGO, G., BOUYSSOU, H. and BELKOURA, M. (1985): Vacoular efflux of malate and its influence on nitrate accumulation in *Cathranthus roseus* cells. — Plant Sci. Lett. 39, 97—103.
- MARGÓCZI, K. and MARÓTI, I. (1985): The spatial distribution of carbohydrates in the leaves of maize grown in various light-dark cycles. — Acta Biol. Szeged 31, 87—96.
- MARÓTI, I. (1982): Effect of short light dark cycles on the chlorophyll and carotenoid content of maize and tomatoes. — Acta Biol. Szeged. 28, 1—4.
- MARÓTI, I. (1986): Genotypical differences in the changes of light adaptation in the structure of leaf chloroplast. — Proceedings of IV. Hungarian Symposium on Plant Anatomy p. 18. Budapest.
- MARÓTI, I. and TAKÁCS, E. (1983): Effect of short periods of light on the organization of the membraneous system of corn mesophyll chloroplasts. — Acta Biol. Szeged. 29, 33—34.
- MARÓTI, I. and MARGÓCZI, K. (1984): Effect of identifical and alternating light dark periods on the growth, dry matter accumulation and carbohydrate content of maize leaves. — Acta Biol. Szeged. 30, 51—59.

- NEYRA, C. and HAGEMANN, R. H. (1976): Relationships between carbon-dioxide, malate and nitrate accumulation and reduction in corn (Zea mays L.) seedlings. — Plant Physiol. 52, 726—730.
- PATAKY, Sz. and MARÓTI, I. (1985): Connection of different photosynthetic activity with the structure of the chloroplast. — Proceedings of Hungarian—Austrian Joint Conference on Electron Microscopy p. 114. Balatonaliga.
- PREISS, J. (1986): Fructose-2,6-bisphosphate: Present status and future prospects. Physiol. Plant. 69, 373—376.
- SCHEIBE, R. WAGENPFEIL, D. and FISHER, J. (1986): NADP-malate-dehydrogenase activity during photosynthesis in illuminated spinach chloroplasts. — J. Plant Physiol. 124, 103—110.
- SLACK, C. R., HATCH, M. D. and GOODCHILD, D. J. (1969): Distribution of enzymes in mesophyll and parenchyma sheath chloroplasts of maize leaves in relation to the C4 dicarboxylic acid pathway of photosynthesis. — Biochem. J. 114, 489—498.
- STITT, M., GERHARDT, R., WILKE, I. and HELDT, H. W. (1987): The contribution of fructose-2,6bisphosphate to the regulation of sucrose synthesis during photosynthesis. — Physiol. Plant. 69, 377—386.
- TECSI, L. (1987): Comparison of photosynthetic performances in the leaves of two maize and bean genotypes in the initial period of light. PhD thesis. József Attila University, Szeged.
- TOLBERT, N. E. (1979): Glycollate metabolism by higher plants and algae. In: GIBBS, M., LATZKO, E. (eds.): Photosynthesis II. Encyclopaedia of Plant Physiology. New Series Vol. 6. Pp. 338—352. Springer Verlag, Berlin.
- WALKER, D. A. (1985): Measurement of oxygen and chlorophyll fluorescence. In: COOMBS, J., HALL, D. O., LONG, S. P., SCURLOCK, J. M. O. (eds.): Techniques in Bioproductivity and Photosynthesis. Pp. 95—106. Pergamon Press, Oxford.