PHOTOSYNTHETICA 37 (1): 113-121, 1999

Leaf gas exchange and chlorophyll fluorescence parameters in *Phaseolus vulgaris* as affected by nitrogen and phosphorus deficiency

J.D. LIMA, P.R. MOSQUIM, and F.M. DA MATTA*

Departamento de Biologia Vegetal, Universidade Federal de Viçosa, 36571-000 Viçosa, MG, Brasil

Abstract

The effects of N and P deficiency, isolated or in combination, on leaf gas exchange and fast chlorophyll (Chl) fluorescence emission were studied in common bean cv. Negrito. 10-d-old plants grown in aerated nutrient solution were supplied with high N (HN, 7.5 mol m^{-3}) or low N (LN, 0.5 mol m^{-3}), and also with high P (HP, 0.5 mol m^{-3}) or low P (LP, 0.005 mol m^{-3}). Regardless of the external P supply, in LN plants the initial fluorescence (F_0) increased 12 % in parallel to a quenching of about 14 % in maximum fluorescence (F_m). As a consequence, the variable to maximum fluorescence ratio (F_v/F_m) decreased by about 7 %, and the variable to initial fluorescence ratio (F_v/F_0) was lowered by 25 % in relation to control plants. In LP plants, F_v/F_m remained unchanged whilst F_v/F_0 decreased slightly as a result of 5 % decline in F_m. Under N deficiency, the net photosynthetic rate (P_N) halved at 6 d after imposition of treatment and so remained afterwards. As compared to LN plants, P_N declined in LP plants latter and to a less extent. From 12 d of P deprivation onwards, P_N fell down progressively to display rates similar to those of LN plants only at the end of the experiment. The greater P_N in LP plants was not reflected in larger biomass accumulation in relation to LN beans. In general, P and N limitation affected photosynthesis parameters and growth without showing any synergistic or additive effect between deficiency of both nutrients.

Additional key words: biomass accumulation; chlorophyll; common bean; fluorescence induction; net photosynthetic rate; stomatal conductance.

Received 16 August 1999, *accepted* 18 October 1999.
*Corresponding author; fax: +55 31 899 2580; e-mail: fdamatta@mail.ufv.br
Acknowledgements: The authors are grateful to the FAPEMIG (Fundação de Amparo ÷ Pesquisa do Estado de Minas Gerais) for financial support to this research. J.D.L. held a scholarship from CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico, Brazil).

Introduction

The net photosynthetic rate (P_N) declines with a drop in leaf N status in many species (Evans 1989, Poorter and Evans 1998). Inadequate N supply in addition to altering stomatal conductance (Hák and Nátr 1987, Ciompi *et al.* 1996, Cechin 1998) may also induce decreases in both chlorophyll (Chl) (Khamis *et al.* 1990, Peñuelas *et al.* 1993) and total soluble protein (Evans 1989, Schäfer and Heim 1992) contents. Under N-limitation a lower proportion of N is allocated to the operative enzymes of the Calvin cycle, most particularly ribulose-1,5-bisphosphate carboxylase/oxygenase, whereas the proportion allocated to thylakoids may not change under different N levels (Evans 1989). That being so, the N-restricted P_N seems to be due chiefly to an impaired capacity of carboxylation rather than to a decline of electron transport and/or photophosphorylation (Tan and Hogan 1995).

Reports concerning the effects of P deficiency on P_N per unit leaf area are somewhat conflicting. In several plant species P deprivation leads to large decreases in P_N (Foyer and Spencer 1986, Jacob and Lawlor 1993) whereas in other species P_N may not be affected (Foyer and Spencer 1986, Crafts-Brandner 1992). This may depend on both the extent of leaf P deficiency and the capability of plant metabolism to cope with low internal P supply, *e.g.*, *via* increasing recirculation of inorganic phosphate (Pi) during glycolic and phosphoenolpyruvate metabolism (Kondracka and Rychter 1997). If P_N is responsive to P starvation, decreases in activity and activation states of some key enzymes of the Calvin cycle are usually invoked to account for low P-limited P_N (Rao and Terry 1989). However, a deficiency of ATP (Jacob and Lawlor 1993) and an increasing diversion of triose phosphate into starch (sometimes also sucrose) at the expense of ribulose-1,5-bisphosphate biosynthesis (Dietz and Harris 1997) may more directly slow the Calvin cycle. Insufficient supply of P may also limit P_N per unit area by altering leaf Chl and protein contents (Plesničar *et al.* 1994, Usuda 1995), but to a lesser degree than often does the N deficiency.

The effects of both N and P on photosynthesis and biomass production are well documented. Little is known, however, how simultaneous deficiency of both elements affects some basic physiological processes. As the declining P_N in response to either N or P deficiency may arise from different causes, additive or synergistic effects of concurrent N and P limitation on P_N are to be expected. This work focused on the effects of N and P deprivation, isolated or in combination, on gas exchange parameters, and Chl fast fluorescence emission in common bean leaves.

Materials and methods

Plants and growth conditions: Four days after emergence (DAE) on sterilised quartz sand, *Phaseolus vulgaris* L. cv. Negrito seedlings were transplanted to individual polystyrene containers lined with a transparent plastic sheet and containing 4 500 cm^3 of half-strength Hoagland's nutrient solution (Hoagland and Arnon 1950) adjusted daily to pH 5.5 and aerated continuously. Plants were grown in a greenhouse under natural irradiance. At 10 DAE, N and P deficiency, isolated or in combination, was

imposed. Plants were then supplied with high N (HN, 7.5 mol m^{-3}) or low N (LN, 0.5 mol m^{-3}), and also with high P (HP, 0.5 mol m^{-3}) or low P (LP, 0.005 mol m^{-3}). Nitrogen was supplied to culture solution as ammonium nitrate in N-deficient plants; the remaining amount to perform the HN supply in other treatments was provided with calcium nitrate and potassium nitrate. Phosphorus was supplied as monopotassium phosphate. Original Hoagland's solution was modified in order to vary N and/or P concentrations whilst keeping an optimal availability of the other nutrients. Solutions were changed every five days; 2 cm^3 Fe-EDTA were added every other day. Root pathogen attack was prevented by adding a 0.1 g m^{-3} suspension of *Metalaxyl* plus *Dithane* to the nutrient solution.

Biochemical assays: Chls were determined according to Hendry and Price (1993). Inorganic phosphate was extracted as described by Hogue *et al.* (1970), and assayed according to Braga and Defelipo (1974). Total N was quantified by the method of Lang (1958). These assays were performed on the youngest, completely expanded central leaflet at the end of the experiment (28 DAE).

Growth was estimated by measuring the total leaf area by an area meter (*Area Measurement System, Delta-T Devices*, Cambridge, U.K.), and the dry mass of shoots and roots.

Photosynthetic parameters were estimated between 09:00 and 11:00 h on the attached youngest, completely expanded central leaflet at 6, 9, 12, 15, and 18 d after imposing the nutritional deficiency. Stomatal conductance to water vapour (g_s), internal to ambient CO_2 concentration ratio (C_i/C_a), and P_N were measured with a portable, open-system infrared gas analyser (*LCA-2, ADC*, Hoddesdon, U.K.) at ambient CO_2 concentration under artificial, saturating photosynthetic photon flux (about 850 μmol m^{-2} s^{-1}) supplied by an accessory for the *ADC* leaf chambers. During the measurements, leaf temperature ranged from 28 to 34 °C. Fast Chl fluorescence emission was estimated using a portable Chl fluorometer (*PEA, Hansatech*, Norfolk, U.K.) in leaves previously adapted to darkness for 30 min at room temperature, as described by Da Matta *et al.* (1997). The initial (F_0) and maximum (F_m) Chl fluorescence were then measured from which the ratios of variable to maximum (F_v/F_m) or to initial (F_v/F_0) fluorescence were calculated.

Experimental design and statistical analysis: Only for the plant material sampled at the end of the experiment (biochemical assays and growth characteristics), the experimental layout was at random with four treatments (two N and two P levels) and five replicates. For the photosynthesis values, the experimental design was in split-plot with the two N and two P levels randomly distributed through the main plots and the times of photosynthetic measurements considered as the subplots. Each experimental plot was constituted by one plant per container. Analysis of variance was used to examine the effects of N and P on the measured parameters. When interactions between the main plots and subplots were significant, partition into the components attributable to N and P effects, isolated or in combination, was performed. Results from decomposition of interactions were presented only when

interactions were significant. Statistical significance among means was analysed by Tukey's test, at $p = 0.05$.

Results and discussion

At 28 DAE, the total Chl content was halved in plants grown in LN, but it was not altered in LP plants (Table 1). Leaf Pi, considered the main fraction of total leaf P which changes in response to P deficiency, was lowered by about 80 % in LP plants as compared to the control ones (Table 1). A relatively smaller decrease was found for leaf N levels in LN and LN+LP plants, possibly as a consequence of the N-fixing symbiont activity and/or a more efficient remobilization of N from older to younger tissues. Alterations in plant nutrient status were reflected on the strong inhibition of shoot growth and total leaf area; root biomass, however, was not affected by deficiency treatments (Table 1). No synergistic effect of N and P deficiency on any of measured growth parameters was observed.

Table 1. Chlorophyll (Chl) (*a*+*b*), total N, and Pi contents, all expressed in mmol m^{-2}, and total leaf area [m^2] and dry mass of both shoots and roots [g] in common bean as determined in the youngest, expanded leaflet 18 d after imposing N and P deficiency. Plants were supplied with high N (HN, 7.5 mol m^{-3}) or low N (LN, 0.5 mol m^{-3}), and also with high P (HP, 0.5 mol m^{-3}) or low P (LP, 0.005 mol m^{-3}). Means followed by the same letter in the column do not differ statistically by the Tuckey's test at $p = 0.05$.

Treatment	Chl (*a*+*b*)	P	N	Leaf area	Dry mass shoot	root
HP+HN	0.40 a	0.72 b	88.05 a	0.37 a	14.4 a	2.9
HP+LN	0.21 b	1.16 a	40.28 b	0.09 b	3.8 b	2.7
LP+HN	0.40 a	0.14 c	92.50 a	0.10 b	4.7 b	3.3
LP+LN	0.22 b	0.14 c	39.72 b	0.09 b	3.7 b	3.1

Chl fluorescence parameters were differently affected by the treatments (Table 2). Plants grown in LN, regardless of the external P supply, exhibited a 12 % increase in F_0 paralleling a quenching in F_m by about 14 %. Altogether, these alterations brought about a 7 % decrease in F_v/F_m ratio, which represents the PS2 photochemical efficiency in the dark-adapted state with fully open PS2 reaction centres. Decreases in F_v/F_m were also found in suspension cultured cells of *Chenopodium rubrum* (Schäfer and Heim 1992) and in maize (Khamis *et al.* 1990) grown under N deprivation. By contrast, no depression in F_v/F_m was found in tobacco (Balachandran and Osmond 1994), sorghum (Cechin 1998), and sunflower (Ciompi *et al.* 1996), even though the total Chl content dropped by about half in the latter. These divergences of N-dependent F_v/F_m behaviour suggest both the different mechanisms to efficiently recycle N within the plant and a potentially different infra-leaf allocation of N. Anyhow, in this report, impairment of the PS2 photochemistry could be better envisaged by the larger decrease (about 25 %) in the F_v/F_0 ratio (Table 2)

which showed a much grater amplitude than F_v/F_m, and hence more sensitively reflected changes in photosynthetic activity. In contrast, the F_v/F_m ratio is relatively inert and slow in response, and does not and cannot respond readily to small changes in F_v or F_0, since F_m is the sum of F_v plus F_0 (Babani and Lichtenthaler 1996). According to these authors, F_m does not change at all, *e.g.*, when F_v slightly decreases and F_0 is slightly increased. Therefore, changes in photosynthetic quantum conversion and photochemical efficiency of PS2 could be masked by forming the F_v/F_m ratio. In any case, disturbances of photosynthetic activity, as judged by the decreased F_v/F_0 ratio, had already started six days after the LN treatment, irrespective of the P supply (Fig. 1*A*). This might have been associated to loss of pigments (Table 1) and also possibly to imbalances in the allocation of assimilates due to depressed growth under N stress (Table 1). As an increase in F_0 with a quenched F_m was observed, damage to D1 protein and other reaction centre components probably should have occurred (Krause and Weis 1991). Because N stress likely reduces the capacity for protein synthesis, photodamaged PS2 centres could not be repaired sufficiently, and then photoinhibition would be manifested (Godde and Hefer 1994). On the other hand, little tendency towards photoinhibition was evident in LP-treated plants. F_v/F_m was not altered, whereas the average F_v/F_0 ratio along with the time was only slightly depressed (Table 2). In strict sense, only at the 9th d after LP treatment F_v/F_0 was significantly lowered as compared to control plants (Fig. 1*A*). The decrease in that ratio was a consequence of a mean 5 % quenching in F_m with an unchanged F_0 (Table 2), indicating a small rise in thermal deactivation of the PS2 reaction centres (Krause and Weis 1991). These results are in consonance with those of Abadía *et al.* (1987), who showed that the primary processes of photochemical reactions of photosynthesis were relatively little affected by P deficiency.

Table 2. Initial (F_0) and maximum (F_m) fluorescence, the ratio of variable to maximum (F_v/F_m) or initial fluorescence (F_v/F_0), stomatal conductance to water vapour (g_s) [mol m^{-2} s^{-1}], internal to ambient CO_2 concentration ratio (C_i/C_a), and net photosynthetic rate (P_N) [μmol(CO_2) m^{-2} s^{-1}] measured in common bean leaves. Fluorescence and gas exchange parameter values are means from values collected 6, 9, 12, 15, and 18 d following imposition of nutrient deficiency. See legend to Table 1 for further details.

Treatment	F_0	F_m	F_v/F_m	F_v/F_0	g_s	C_i/C_a	P_N
HP+HN	578 c	3200 a	0.818 a	4.50 a	0.39 a	0.649 c	15.79 a
HP+LN	648 ab	2731 b	0.760 b	3.31 c	0.23 b	0.714 b	7.44 c
LP+HN	606 bc	3055 a	0.801 a	4.08 b	0.27 b	0.663 bc	11.06 b
LP+LN	652 a	2800 b	0.766 b	3.36 c	0.31 ab	0.778 a	7.13 c

P_N values were decreased to a greater extent in both LN and LN+LP plants than in LP plants, but no additive effect of combined N and P deficiency on P_N was observed (Table 2). Under N starvation, P_N had been already halved at 6 d after imposing the deficiency, not showing any further considerable decrease until the end of the experiment (Fig. 1*B*). The reduced P_N was accompanied by a 42 % depression in g_s in LN plants, and by a non-significant 21 % decrease in g_s in LN+LP plants

(Table 2). It is unlikely, however, that g_s had restrained P_N since: (*1*) C_i/C_a increased,

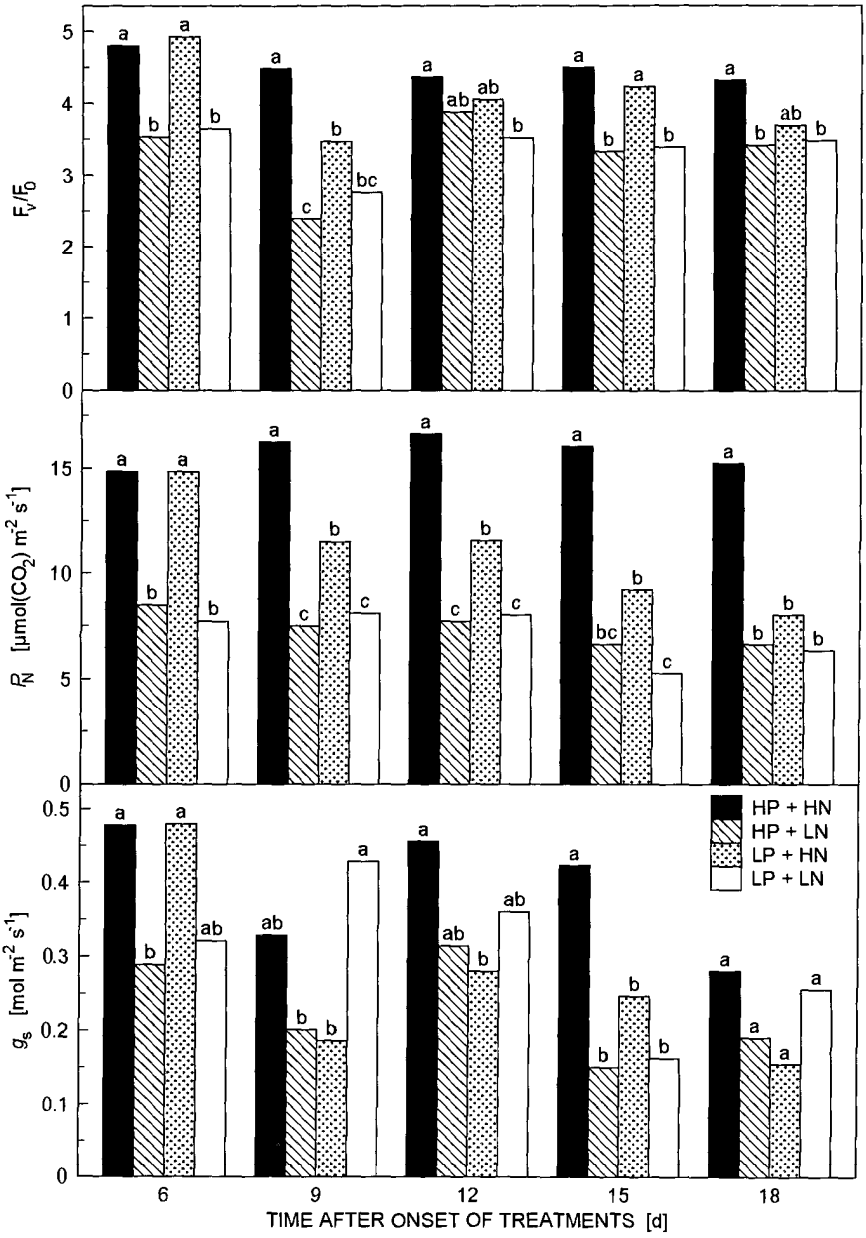

Fig. 1. (*A*) Variable to initial fluorescence ratio (F_v/F_0), (*B*) stomatal conductance to water vapour (g_s), and (*C*) net photosynthetic rate (P_N) in common bean as determined on the youngest, expanded leaflet along with the imposition of N and P deficiency. Plants were supplied with high N (HN, 7.5 mol m^{-3}) or low N (LN, 0.5 mol m^{-3}), and also with high P (HP, 0.5 mol m^{-3}) or low P (LP, 0.005 mol m^{-3}). Bars followed by the same letter do not differ statistically at a given time, by the Tuckey's test at $p = 0.05$.

especially in LN+LP plants (Table 2); (*2*) g_s of nutrient deficient plants on several occasions was greater than 0.29 mol m^{-2} s^{-1}, a value able to support high P_N, as observed in control plants at the end of the experiment (Fig. 1*C*); and (*3*) assessment of P_N with an oxygen electrode at 5 % CO_2, which is a benchmark reflecting minimal or no limitation to diffusion of CO_2 from outside atmosphere until the carboxylation sites, revealed a decrease in photosynthetic capacity similar to that reported here (not shown). Therefore, these results suggest that biochemical constraints, rather than stomatal effects, constituted the predominant limitations to photosynthesis.

The rapid, substantial decrease in P_N per unit leaf area (Fig. 1*B*) under N deficiency probably reflects a rapid drop in plant N contents (Robinson 1996). In tobacco, for instance, P_N and activity and amount of ribulose-1,5-bisphosphate carboxylase/oxygenase declined remarkably after omission of N from the culture solution for 3 d, even though Chl content was not altered until 8 d after omission of N (Paul and Driscoll 1997). In this work, declines in both enzyme amounts, as suggested by the decreases in total leaf N, and Chl content (Table 1) should have greatly contributed to impairing P_N. It is possible that PS2 photochemistry has not considerably affected carbon gain, as the decreases in both F_v/F_m and F_v/F_0 ratios were not so large under N starvation (Table 2). On the contrary, the failure in maintaining a high, normal PS2 efficiency might have been a consequence, and not a cause, of the partial loss of the photosynthetic capacity.

As mentioned earlier, P deficiency was less effective than N deprivation in causing a decline in P_N. Plants grown in LP exhibited an average 26 % decrease in P_N (Table 2), and such decrease was observed only 9 d after induction of P limitation (Fig. 1*B*). After 12 d of P deficiency, P_N was progressively depressed, showing a similar magnitude to that of N-deficient plants only at the end of the experiment. Decreases of P_N in LP plants, in spite of being accompanied by a 31 % lowering in g_s (Table 2), were a result of biochemical limitations. This suggestion is supported by the same reasons invoked to dismiss stomatal limitation to P_N in plants grown under LN. Conversely to these plants, LP beans neither showed any decline in Chl content nor in leaf N concentration (Table 1), the latter being not uncommonly decreased under P starvation (Jeschke *et al.* 1997, Gniazdowska *et al.* 1999). Thus, the reduction in P_N seemed to be directly triggered by the strong decline in leaf Pi in LP plants (Table 1). According to Loughman *et al.* (1989), Pi in both cytosol and chloroplast decreases progressively under P deprivation until reaching a limiting concentration that restrains P_N. In the initial phases of deficiency, the vacuole may buffer against fluctuations in Pi levels in the cytosol (Dietz and Harris 1997). This might explain why P_N fell down in LP plants only after 9 d of P limitation.

Although P_N was considerably less impaired in LP plants, they did not grow better than LN or LN+LP plants. In effect, the plants under mineral deprivation exhibited a similar biomass and total leaf area (Table 1). Moreover, growth was depressed to a greater magnitude than was P_N. Altogether, these results thus evidence that the restricted P_N as a response to nutrient deficiency was not the direct cause of decreased biomass accumulation. According to Dietz and Harris (1997), inhibition of P_N may be due directly to an increasing leaf assimilate content under nutrient deficiency. This in turn suggests that such inhibition may to a certain extent reflect

a downregulation of P_N owing to excess source activity as compared to sink requirements. Nonetheless, all the above relations must be cautiously envisaged, as growth necessarily integrates complex physiological and morphological changes along with the time, whereas instantaneous measurements of photosynthesis parameters from single leaves express a momentary plant performance, in addition to extrapolating them to the whole plant.

References

Abadía, J., Rao, I.M., Terry, N.: Changes in leaf phosphate status have only small effects on the photochemical apparatus of sugar beet leaves. - Plant Sci. **50**: 49-55, 1987.

Babani, F., Lichtenthaler, H.K.: Light-induced and age-dependent development of chloroplasts in etiolated barley leaves as visualized by determination of photosynthetic pigments, CO_2 assimilation rates and different kinds of chlorophyll fluorescence ratios. - J. Plant Physiol. **148**: 555-566, 1996.

Balachandran, S., Osmond, C.B.: Susceptibility of tobacco leaves to photoinhibition following infection with two strains of tobacco mosaic virus under different light and nitrogen nutrition regimes. - Plant Physiol. **104**: 1051-1057, 1994.

Braga, J.M., Defelipo, B.V.: [Spectrophotometric determination of phosphorus in plant and soil extracts.] - Rev. Ceres **21**: 73-85, 1974. [In Port.]

Cechin, I.: Photosynthesis and chlorophyll fluorescence in two hybrids of sorghum under different nitrogen and water regimes. - Photosynthetica **35**: 233-240, 1998.

Ciompi, S., Gentili, E., Guidi, L., Soldatini, G.F.: The effect of nitrogen deficiency on leaf gas exchange and chlorophyll fluorescence parameters. - Plant Sci. **118**: 177-184, 1996.

Crafts-Brandner, S.J.: Phosphorus nutrition influence on leaf senescence in soybean. - Plant Physiol. **98**: 1128-1132, 1992.

Da Matta, F.M., Maestri, M., Mosquim, P.R., Barros, R.S.: Photosynthesis in coffee (*Coffea arabica* and *C. canephora*) as affected by winter and summer conditions. - Plant Sci. **128**: 43-50, 1997.

Dietz, K.-J., Harris, G.C.: Photosynthesis under nutrient deficiency. - In: Pessarakli, M. (ed.): Handbook of Photosynthesis. Pp. 951-975. Marcel Dekker, New York - Basel - Hong Kong 1997.

Evans, J.R.: Photosynthesis and nitrogen relationships in leaves of C_3 plants. - Oecologia **78**: 9-19, 1989.

Foyer, C., Spencer, C.: The relationship between phosphate status and photosynthesis in leaves. Effects of intracellular orthophosphate distribution, photosynthesis and assimilate partitioning. - Planta **167**: 369-375, 1986.

Gniazdowska, A., Krawczak, A., Mikulska, M., Rychter, A.M.: Low phosphate nutrition alters bean plants ability to assimilate and translocate nitrate. - J. Plant Nutr. **22**: 551-563, 1999.

Godde, D., Hefer, M.: Photoinhibition and light-dependent turnover of the D1 reaction-centre polypeptide of photosystem II are enhanced by mineral-stress conditions. - Planta **193**: 290-299, 1994.

Hák, R., Nátr, L.: Effect of nitrogen starvation and recovery on gas exchange characteristics of young barley leaves. - Photosynthetica **21**: 9-14, 1987.

Hendry, G.A.F., Price, J.P.: Stress indicators: chlorophylls and carotenoids. - In: Hendry, G.A.F., Grime, A.H. (ed.): Methods in Comparative Plant Ecology. Pp. 148-152. Chapman & Hall, London 1993.

Hoagland, D.R., Arnon, D.I.: The water culture method for growing plants without soil. - California Agric. exp. Sta. Circ. **347**: 1-32, 1950.

Hogue, E., Wilcox, G.E., Cantliffe, D.J.: Effect of soil phosphorus levels on phosphate fractions in tomato leaves. - J. amer. Soc. hort. Sci. **95**: 174-176, 1970.

Jacob, J., Lawlor, D.W.: Extreme phosphate deficiency decreases the *in vivo* CO_2/O_2 specificity factor of ribulose-1,5-bisphosphate carboxylase-oxygenase in intact leaves of sunflower. - J. exp. Bot. **44**: 1635-1641, 1993.

Jeschke, W.D., Kirkby, E.A., Peuke, A.D., Pate, J.S., Hartung, W.: Effects of P deficiency on assimilation and transport of nitrate and phosphate in intact plants of castor bean (*Ricinus communis* L.). - J. exp. Bot. **48**: 75-91, 1997.

Khamis, S., Lamaze, T., Lemoine, Y., Foyer, C.: Adaptation of the photosynthetic apparatus in maize leaves as a result of nitrogen limitation. Relationships between electron transport and carbon assimilation. - Plant Physiol. **94**: 1436-1443, 1990.

Kondracka, A., Rychter, A.M.: The role of Pi recycling processes during photosynthesis in phosphate-deficient plants. - J. exp. Bot. **48**: 1461-1468, 1997.

Krause, G.H., Weis, E.: Chlorophyll fluorescence and photosynthesis: The basics. - Annu. Rev. Plant Physiol. Plant mol. Biol. **42**: 313-349, 1991.

Lang, C.A.: Simple microdetermination of Kjeldahl nitrogen in biological materials. - Anal. Chem. **30**: 1692-1692, 1958.

Loughman, B.C., Ratcliffe, R.G., Southon, T.E.: Observations on the cytoplasmic and vacuolar orthophosphate pools in leaf tissues using *in vivo* ^{31}P-NMR spectroscopy. - FEBS Lett. **242**: 279-284, 1989.

Paul, M.J., Driscoll, S.P.: Sugar repression of photosynthesis: the role of carbohydrates in sinalling nitrogen deficiency through source:sink imbalance. - Plant Cell Environ. **20**: 110-116, 1997.

Peñuelas, J., Biel, C., Estiarte, M.: Changes in biomass, chlorophyll content and gas exchange of beans and peppers under nitrogen and water stress. - Photosynthetica **29**: 535-542, 1993.

Plesničar, M., Kastori, R., Petrović, N., Panković, D.: Photosynthesis and chlorophyll fluorescence in sunflower (*Helianthus annuus* L.) leaves as affected by phosphorus nutrition. - J. exp. Bot. **45**: 919-924, 1994.

Poorter, H., Evans, J.R.: Photosynthetic nitrogen-use efficiency of species that differ inherently in specific leaf area. - Oecologia **116**: 26-37, 1998.

Rao, I.M., Terry, N.: Leaf phosphate status, photosynthesis, and carbon partitioning in sugar beet. I. Changes in growth, gas exchange, and Calvin cycle enzymes. - Plant Physiol. **90**: 814-819, 1989.

Robinson, J.M.: Leaflet photosynthesis rate and carbon metabolite accumulation patterns in nitrogen-limited, vegetative soybean plants. - Photosynth. Res. **50**: 133-148, 1996.

Schäfer, C., Heim, R.: Nitrogen deficiency exacerbates the effects of light stress in photoautotrophic suspension cultured cells of *Chenopodium rubrum*. - Photosynthetica **27**: 545-561, 1992.

Tan, W., Hogan, G.D.: Limitations to net photosynthesis as affected by nitrogen status in jack-pine (*Pinus banksiana* Lamb.) seedlings. - J. exp. Bot. **46**: 407-413, 1995.

Usuda, H.: Phosphate deficiency in maize. V. Mobilization of nitrogen and phosphorus within shoots of young plants and its relationship to senescence. - Plant Cell Physiol. **36**: 1041-1049, 1995.