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

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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 (F₀) increased 12 % in parallel to a quenching of about 14 % in maximum fluorescence (Fₘ). As a consequence, the variable to maximum fluorescence ratio (Fₐ/Fₘ) decreased by about 7 %, and the variable to initial fluorescence ratio (Fₐ/F₀) was lowered by 25 % in relation to control plants. In LP plants, Fₐ/Fₘ remained unchanged whilst Fₐ/F₀ decreased slightly as a result of 5 % decline in Fₘ. Under N deficiency, the net photosynthetic rate (Pₙ) halved at 6 d after imposition of treatment and so remained afterwards. As compared to LN plants, Pₙ declined in LP plants latter and to a less extent. From 12 d of P deprivation onwards, Pₙ fell down progressively to display rates similar to those of LN plants only at the end of the experiment. The greater Pₙ 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.

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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 Amon 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 ($C_l/C_a$), 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 \([\text{m}^2]\) and dry mass of both shoots and roots \([\text{g}]\) 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 \( p = 0.05 \).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Chl ((a+b))</th>
<th>P</th>
<th>N</th>
<th>Leaf area</th>
<th>Dry mass</th>
<th>root</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>shoot</td>
<td>shoot</td>
<td>root</td>
</tr>
<tr>
<td>HP+HN</td>
<td>0.40 (\text{a})</td>
<td>0.72 (\text{b})</td>
<td>88.05 (\text{a})</td>
<td>0.37 (\text{a})</td>
<td>14.4 (\text{a})</td>
<td>2.9</td>
</tr>
<tr>
<td>HP+LN</td>
<td>0.21 (\text{b})</td>
<td>1.16 (\text{a})</td>
<td>40.28 (\text{b})</td>
<td>0.09 (\text{b})</td>
<td>3.8 (\text{b})</td>
<td>2.7</td>
</tr>
<tr>
<td>LP+HN</td>
<td>0.40 (\text{a})</td>
<td>0.14 (\text{c})</td>
<td>92.50 (\text{a})</td>
<td>0.10 (\text{b})</td>
<td>4.7 (\text{b})</td>
<td>3.3</td>
</tr>
<tr>
<td>LP+LN</td>
<td>0.22 (\text{b})</td>
<td>0.14 (\text{c})</td>
<td>39.72 (\text{b})</td>
<td>0.09 (\text{b})</td>
<td>3.7 (\text{b})</td>
<td>3.1</td>
</tr>
</tbody>
</table>

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 \(F/F_m\) ratio, which represents the PS2 photochemical efficiency in the dark-adapted state with fully open PS2 reaction centres. Decreases in \(F/F_m\) 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 \(F/F_m\) was found in tobacco (Balachandran and Osmond 1994), sorghum (Cechin 1998), and sunflower (Ciompì et al. 1996), even though the total Chl content dropped by about half in the latter. These divergences of N-dependent \(F/F_m\) 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 \(F/F_0\) ratio (Table 2)
which showed a much greater 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. 1A). 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. 1A). 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 Abadia 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 (\( P_N \)) [\( \mu \text{mol}(\text{CO}_2)\) 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.

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

\( P_N \) 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. 1B). 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 ($F_v/F_o$), (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$^{-2}$) or low N (LN, 0.5 mol m$^{-2}$), and also with high P (HP, 0.5 mol m$^{-2}$) or low P (LP, 0.005 mol m$^{-2}$). Bars followed by the same letter do not differ statistically at a given time, by the Tuckey's test at $p = 0.05$. 

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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. 1C); 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. 1B) 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. 1B). 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 \( P_i \) in LP plants (Table 1). According to Loughman et al. (1989), \( P_i \) 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 \( P_i \) 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


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.


