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Photosynthetic net O_2 evolution enhancement as a sign of acclimation to phosphorus deficiency in bean *(Phaseolus vulgaris* L.) leaves

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Abstract

Primary leaves of bean (*Phaseolus vulgaris* L.) seedlings cultivated for 14 days in a growth chamber on complete (control) and phosphate deficient (–P) Knop liquid medium were used for measurements. The –P leaves were smaller and showed an increased specific leaf area (SLA). Their inorganic phosphate (P_i) concentration was considerably lowered. They did not show any significant changes in chlorophyll (Chl) ($a + b$) concentration and in their net CO_2 assimilation rate when it was estimated under the conditions close to those of the seedlings growth. Light response curves of photosynthetic net O_2 evolution (P_NO_2) of the leaves for the irradiation range up to 500 µmol(photon) m^{-2} s^{-1} were determined, using the leaf-disc Clark oxygen electrode. The measurements were taken under high CO_2 concentration of about 1 % and O_2 concentrations of 21 % or lowered to about 3 % at the beginning of measurement. The results obtained at 21 % O_2 and the irradiations close to or higher than those used during the seedlings growth revealed the phosphorus stress suppressive effect on the leaf net O_2 evolution, however, no such effect was observed at lower irradiations. Other estimated parameters of P_NO_2 such as: apparent quantum requirement (QR_A) and light compensation point (LCP) for the control and –P leaves were similar. However, with a high irradiation and lowered O_2 concentration the rate of P_NO_2 for the –P leaves was markedly higher than that for the control, in relation to both the leaf area and leaf fresh mass. This difference also disappeared at low irradiations, but the estimated reduced QR_A values indicate, under those conditions, the increased yield of photosynthetic light reaction, especially in the –P leaves. The presented results confirm the suggestion that during the initial phase of insufficient phosphate feeding the acclimations in the light phase of photosynthesis, both structural and functional appear. They correspond, probably, to the increased energy costs of carbon assimilation under phosphorus stress, *e.g.* connected with raised difficulties in phosphate uptake and turnover and enhanced photorespiration. Under the experimental conditions especially advantageous for the dark phase of photosynthesis (saturating CO_2 and PAR, low O_2 concentration), those acclimations may be manifested as an enhancement of photosynthetic net O_2 evolution.

Additional key words: Mehler reaction; photosynthetic net O_2 evolution; *Phaseolus vulgaris*; phosphorus deficiency; photorespiration; quantum requirement.

Introduction

Insufficient inorganic phosphate (P_i) feeding affects plant development and ultimately reduces photosynthetic carbon assimilation, growth and productivity. Suboptimal P_i plant status reduces photosynthesis mainly by the limitation of RuBP regeneration (Rychter and Rao 2005), whereas the photochemical apparatus and light reactions are only slightly affected (Brooks 1986, Abadia *et al.* 1987, Furbank *et al.* 1987, Jacob and Lawlor 1993). P_i deficiency may also influence the photosynthesis indirectly, affecting in the first place the growth processes and the demand for assimilates (Halsted and Lynch 1996, Pieters *et al.* 2001).

During the initial stage of limited P_i availability, various structural and metabolic acclimations are undoubtedly induced, which can neutralize, limit or even eliminate the unfavorable effects of phosphorus stress. It was also previously shown that primary leaves of bean (*Phaseolus vulgaris* L.) seedlings (Kondracka and Rychter 1997) as well as *Chlorella vulgaris* cells (Kozłowska and Maleszewski 1994, Kozłowska-Szerenos *et al.* 2000)

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Abbreviations: Chl − chlorophyll; FM − fresh mass; LCP − light compensation point; PAR − photosynthetically active radiation; P_i − inorganic phosphate; P_N − net CO_2 assimilation rate; P_NO_2 − photosynthetic net O_2 evolution; PPFD − photosynthetic photon flux density; QR_A − apparent quantum requirement of O_2 evolution (estimation based upon incident photon flux density and net O_2 evolution); R_D − dark respiration; SLA − specific leaf area.

acclimated to phosphate deficiency, demonstrated signs of enhanced glycolate photorespiratory metabolism.

The aforenamed acclimation, which increases the energy demand of photosynthesis, may be effective only if the insufficient P_i feeding does not limit the potential activity of light reaction. The results of our previous work with *Chlorella vulgaris* cells confirmed such possibility. Cells from cultures grown in phosphate deficit medium showed an increased potential capacity (higher P_{Nmax} and $1/K_{0.5}$ [dissolved inorganic carbon]) for net photosynthetic oxygen evolution. They utilized more of the absorbed PAR energy for nitrate reduction than the control cells. Under the conditions of more efficient CO_2 supply (high inorganic carbon concentration, the presence of exogenous carbonic anhydrase in the cell suspension) and at a high irradiance the net photosynthetic O_2 evolution of cells from –P cultures reached even higher rate than in the controls (Kozłowska-Szerenos *et al.* 2004).

In the present study the P_NO_2 in the primary leaves of bean *(Phaseolus vulgaris* L.*)* seedlings, well supplied with phosphate (control) or phosphate deficient (–P) were examined. The measurements were taken under the conditions favorable to the dark reaction of photosynthesis (high CO_2, lowered O_2 concentrations). The conditions used allowed the acclimations in the potential ability of photosynthetic light reactions, expressed as enhancement of the photosynthetic net O_2 evolution, to manifest itself in leaves at the early stage of phosphorus stress.

Materials and methods

Plants and growth conditions: The bean *(Phaseolus vulgaris* L., cv. Golden Saxa*)* seedlings were grown in a growth chamber on an aerated Knop mineral liquid medium, pH 6.8, complete (control) or phosphate deficient (–P). Radiation sources of the photosynthetic photon flux density about 200 µmol(photon) m^{-2} s^{-1} were fluorescent tubes *Cool White + DayLight* in the ratio of 1:1 *(Philips Lightening,* Bielsko Biała, Poland*)*. Photoperiod was 16 h, day/night temperature 25/20 °C, and day/night air humidity 65–70/80–90 %. For all measurements leaf discs of the surface of 10 cm^2 were cut out from central parts of the primary leaves of the 14-d-old seedlings. Chlorophylls were extracted from the leaf-discs with 80 % acetone and determined spectrophotometrically (*CE 2501, CECIL Instruments*, Cambridge, England) according to Wellburn (1994). The leaf content of inorganic phosphate was determined with the Ames method (1966).

Measurements of the net CO_2 exchange in leaves were taken using the *LI-COR* CO_2/H_2O analyser (*LI-COR Inc.*, Lincoln, Nebraska, USA) under the conditions close to those for seedling growth (PPFD, 200 µmol(photon) m^{-2} s^{-1}, O_2 and CO_2 at the atmospheric concentrations, temperature 25 °C).

Measurements of the net O_2 exchange in leaves were performed using the *Hansatech* leaf-disc oxygen electrode (Delieu and Walker 1981, Walker 1990, 1992). The irradiation, supplied with 100 W tungsten halogen bulbs, was regulated with neutral density filters (*Hansatech Instruments Ltd*) and measured with phyto-photometer *FF-01* (*SOMOPAN*, Kraków, Poland). In the reaction chamber (*LD1/2, Hansatech*, King`s Linn, Great Britain) used, the CO_2 concentration was higher than 1 % (in equilibrium with 0.2 ml of 1 M $NaHCO_3$ water solution), temperature was 25 °C, and respective O_2 concentration at the beginning of measurements was 21 % or about 3 % (the composition of air and pure nitrogen in the ratio of 1:6).

Rates of net O_2 exchange of leaf-discs were measured under PPFD 500, 250, 100, 53, 33 and 30 µmol(photon) m^{-2} s^{-1} (P_NO_2) and at dark (dark respiration, R_D). Light compensation point (LCP) and apparent quantum requirement (QR_A) were estimated according to Walker (1990), on the basis of the results of net O_2 exchange measurements under PPFD within the range of 100 – 0 µmol(photon) m^{-2} s^{-1}.

Results

Insufficient phosphate feeding caused a considerable lowering of the leaf P_i concentration and a significant decrease of length, breadth and thickness (increase of the specific leaf area, SLA) of the primary leaves of seedlings. Chl (*a* + *b*) content in relation to the leaf area (mg m^{-2}) did not display significant changes but in relation to the leaf fresh mass (FM) the increasing tendency of this parameter appeared, corresponding to the SLA increase. Modification due to phosphorus deficiency did not, however, cause significant changes in the net CO_2 assimilation rate (P_N) when estimated under conditions close to those used during the seedlings growth (Table 1).

Light response curves of net photosynthetic O_2 evolution for the primary leaves of seedlings, grown at PPFD of 200 µmol(photon) m^{-2} s^{-1}, under the conditions of measurements (CO_2 concentration > 1 %, O_2 initial concentrations 21 % or about 3 %, temperature 25 °C) did not reach apparently the maximum level up to the irradiation as high as 500 µmol (photon) m^{-2} s^{-1} (Fig. 1). The results achieved at O_2 concentration close to 21 % and irradiations higher than that applied during the growth of plants revealed some reducing effect of limited phosphate feeding on P_NO_2. At the irradiations close to

Table 1. Characteristics of primary leaves of bean (*Phaseolus vulgaris* L., var. Golden Saxa) seedlings grown on phosphate sufficient (control) and phosphate deficient (–P) medium for 14 days. Chl − chlorophyll, P_N − net CO_2 assimilation rate, SLA − specific leaf area, Means ± SD (n = 3). * Measured under the conditions close to that used during seedling growth.

Parameters	Control	–P	–P [% of control]
Leaf P_i concentration [mg(P_i) g^{-1}(FM)]	0.53 ± 0.06	0.09 ± 0.09	17
Leaf length [cm]	8.6 ± 0.9	6.6 ± 1.04	77
Leaf breadth [cm]	6.6 ± 1.4	5.0 ± 1.04	76
SLA [cm^2 g^{-1}(FM)]	42.9 ±1.9	48.5 ± 1.6	113
Chl(a+b) content			
[mg m^{-2}]	353 ± 52	360 ± 17	102
[mg g^{-1}(FM)]	1.5 ± 0.2	1.7 ± 0.4	115
P_N			
[µmol(CO_2) m^{-2} s^{-1}]	4.8 ± 0.5	4.6 ± 0.7	96
[µmol(CO_2) g^{-1}(FM) min^{-1}	1.2 ± 0.03	1.3 ± 0.2	105

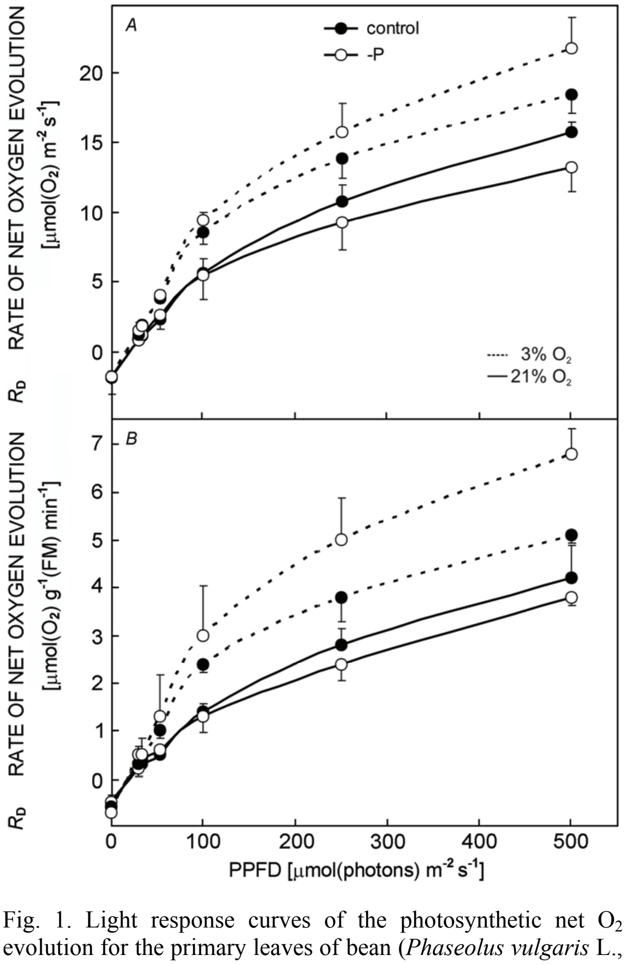

Fig. 1. Light response curves of the photosynthetic net O_2 evolution for the primary leaves of bean (*Phaseolus vulgaris* L., *var*. Golden Saxa) seedlings grown during 14 days on phosphate sufficient (control) and phosphate deficient medium (-P). Results obtained at O_2 concentrations 21 % or about 3% (starting), expressed per leaf area (*A*) and leaf fresh mass (*B*). Means ± SD (n = 3).

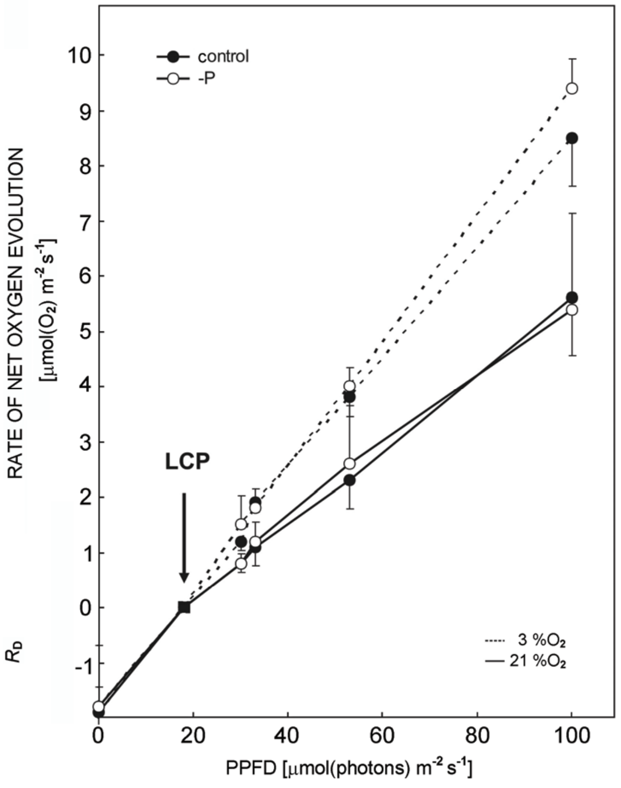

Fig. 2. Near linear parts of the light response curves of the photosynthetic net O_2 evolution for the primary leaves of bean (*Phaseolus vulgaris* L., *var*. Golden Saxa) seedlings grown during 14 days on phosphate sufficient (control) and phosphate deficient medium (–P). Results obtained at O_2 concentrations 21 % or about 3 % (starting) used for evaluation of QR_A and LCP of the photosynthetic net O_2 evolution. Means ± SD (n = 3).

Table 2. Apparent quantum requirement (QR_A) of photosynthetic net O_2 evolution in the primary leaves of bean (*Phaseolus vulgaris* L., var. Golden Saxa) seedlings grown on phosphate sufficient (control) and phosphate deficient (–P) medium for 14 days, estimated from the mean results presented on Fig. 2. QR_A was calculated according to Walker (1990) on the basis of the mean rates of net O_2 evolution measured for PPFD range 30 – 100 μmol(photon) m^{-2} s^{-1} (Fig. 2). Light compensation point (LCP), estimated on the basis of the same result was always close to 17 μmol(photon) m^{-2} s^{-1} (Fig. 2).

O_2 concentration at the start of measurements [%]	QR_A [moles of incident PPFD per mol net O_2 evolved]	
	Control	–P
21	14.6	15.4
3	9.7	8.8

those applied during the experimental plants growth and lower this effect was insignificant or it was not observed (Fig. 1).

Light response curves of P_NO_2 illustrating the results of assays started with the initial O_2 concentration lowered to about 3 %, in comparison with the results obtained at 21 % O_2 demonstrated the enhancement of the net O_2 evolution rates for the leaves of both groups of plants (control and –P). The unexpected feature of this phenomenon, most evident under the highest irradiance used, is its significantly larger size in the leaves of plants under phosphorus stress (Fig. 1).

The light response curves obtained for both groups of experimental plants revealed close to the linear relationship of the net O_2 exchange *vs.* PPFD in the range from 100 down to about 30 μmol(photon) m^{-2} s^{-1}. This allowed estimation of QR_A and LCP of the leaf P_NO_2. These parameters assayed on the basis of measurements carried out at the atmospheric O_2 concentration (Fig. 2, Table 2) did not show significant differences between the control and phosphate deficient leaves. However, stimulation of P_NO_2 in the leaves of –P seedlings, noted in the measurements with the initial O_2 concentration decreased to about 3 %, was connected with the markedly lower value of the apparent quantum requirement /higher value of the apparent quantum yield (Fig. 2, Table 2).

Our measurements did not show a distinct effect of the phosphate feeding on the dark O_2 uptake/respiration (R_D) of leaves, as assayed during 5 min period immediate after the end of irradiation (Figs. 1,2).

Discussion

Direct evidence of phosphorus stress in the primary leaves of –P seedlings used, is a marked decrease of their inorganic phosphate (P_i) content, similar to that found in the earlier studies (Ciereszko *et al.* 1996, Hauschild *et al.* 1996, Kondracka and Rychter 1997). Such a state of –P leaves is confirmed by their decreased length and breadth as well as the increased SLA index (Table 1). The expected effect of the phosphorus stress in their tissues was also a certain decrease of P_NO_2, noted under the conditions of 21 % O_2 and irradiances close to or higher than that used during the seedlings growth (Fig.1).

The experiments concerned the early phase of insufficient phosphate feeding during which the acclimation processes induced were probably already effective whereas functional disturbances due to the phosphorus stress could still be limited. The factor markedly affecting the results was also the atmosphere composition inside the Clark electrode reaction chamber. The high CO_2 concentration used eliminated surely the RuBP oxygenation (Badger *et al.* 2000, von Caemmerer and Quick 2000) and the photorespiratory metabolism of glycolate. It stimulated the assimilation and reduction of CO_2, also at the expense of other pathways competing for the products of photosynthetic light phase, including the Mehler reaction.

P_NO_2 depends on gross O_2 production by the PSII and on the O_2 uptake in the Mehler reaction, glycolate pathway and mitochondrial respiration. It may be reasonably assumed that in the experimental variant with the O_2 initial concentration lowered to about 3 % the Mehler reaction which requires a higher level of O_2 for activity (Furbank and Badger 1983, Miyake *et al.* 1998, Ruuska *et al.* 2000) was also suppressed (Badger *et al.* 2000, Heber 2002). In the water-water cycle, which includes the Mehler reaction, water is oxidized and electrons flow through PSII and PSI to oxygen producing water (Asada 1999, Heber 2002). Under natural conditions the O_2 reduction in the water-water cycle competes with other processes accepting the electrons supplied by the PSII and when it is replaced by those processes its suppression may be expressed by the enhancement of the measured P_NO_2. Such a mechanism may possibly explain the stimulation of the photosynthetic net O_2 evolution (Fig. 1) as well as the decrease of the QR_A (Table 2) at a lowered O_2 concentration both in the control as well as in –P leaves. If this is true, then the increased stimulation of P_NO_2 in the leaves of seedlings acclimated to the phosphate deficiency, noted under the conditions of a lowered O_2 and high CO_2 concentration and under a high irradiation, confirms the suggestion that their photosynthetic apparatus shows a greater potential activity of light phase reactions than the apparatus of the control leaves.

In the irradiations range of below 100 μmol (photon) m^{-2} s^{-1}, where the relation between the net O_2 evolution and irradiations was close to linear, *i.e.* when the incident PPFD was the factor limiting photosynthesis, the inhibitory effect of the phosphate deficiency was not stated. Those results and the QR_A and LCP values estimated on their basis, similar for leaves of the

–P seedlings and the controls, are concordant with generally accepted opinion concerning the lack of a marked effect of phosphate deficiency on the reactions of the light phase of photosynthesis (Abadia *et al.* 1987, Jacob and Lawlor 1993, Rychter and Rao 2005).

The visible decrease in P_NO_2 in leaves with phosphorus deficiency under the conditions of 21 % O_2 and irradiance close to the saturating one may be indirectly related to the inhibitory effect of the P_i deprivation on the dark reactions of the CO_2 assimilation. Inhibition of those reactions may enhance O_2 reduction in the Mehler reaction, which could be expressed by the decrease of the measured P_NO_2 in leaves. On the other hand, under the conditions of O_2 concentration lowered to those inhibiting the Mehler reaction, the reduction of another electron acceptors: nitrate (Champigny 1995), sulfate (Ort and Baker 2002), monodehydroascorbate (Miyake and Asada 1992, Smirnoff 1996), oxaloacetate (Backhausen *et al.* 1998, Cornic and Fresneau 2002), as well as the synthesis of fatty acids (Willms 1999) are undoubtedly favored. Due to synthesis, accumulation and excretion (Kozłowska-Szerenos *et al.* 2004) of products P_NO_2 reaches a higher rate, closer to the gross oxygen production in PSII. This increase of P_NO_2 in the –P seedlings makes visible the effect of acclimation enhancing the potential activity of the photosynthetic light reaction occurring in the early phase of the phosphate deficiency.

Assuming that at a lowered O_2 concentration the O_2 reduction in Mehler reaction is significantly limited and replaced by other sinks of electrons, its involvement in the total photosynthetic electron transport occurring at an atmospheric O_2 concentration may be estimated on the basis of the increment of the photosynthetic O_2 evolution under those conditions. When analysing the results presented in Fig. 1 concerning the highest irradiations (500 µmol(photon) m^{-2} s^{-1}) it may be stated that the increase of P_NO_2, caused by a lowered O_2 concentration to 3 %, in the measurements with the –P leaves was over three times higher than with the control leaves. It seems that this ratio defines the relation of intensity of the Mehler reaction between the –P and the control leaves occurring at 21 % O_2.

The presented results confirm the suggestion that during the early stage of phosphorus stress in the bean leaves there occur acclimations augmenting the potential activity of the light phase of photosynthesis. Probably, their range corresponds to the higher total energy costs of the CO_2 assimilation. Simultaneously, the photochemical quenching mechanisms are increased, which during the intensive irradiation makes possible the safe dispersion of the excess energy, *e.g.* induction of a high activity of the Mehler ascorbate peroxidase pathway (Ort and Baker 2002). Under a low irradiation, when the photosynthesis is limited by the PAR supply, the insufficient phosphate feeding, reducing directly mainly the dark phase (Rychter and Rao 2005), may have no effect on the rate of the photosynthetic net O_2 evolution. However, this acclimation elicited as an enhancement of the net evolution rate under the conditions of saturating irradiance and CO_2, favorable for the reaction of the dark phase of photosynthesis, and of a low O_2 concentration, suppressing O_2 reduction in the Mehler reaction.

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