BIOLOGIA PLANTARUM 48 (3): 401-405, 2004

Changes induced by low oxygen concentration in photosynthetic and respiratory CO_2 exchange in phosphate-deficient bean leaves

S. MALESZEWSKI*, I. CIERESZKO, A. SKOWROŃSKA, E. MIECZEJKO and B. KOZŁOWSKA-SZERENOS

Institute of Biology, University of Białystok, Świerkowa 20B, PL-15950 Białystok, Poland

Abstract

Effect of phosphorus deficiency on photosynthetic and respiratory CO_2 exchanges were analysed in primary leaves of 2-week-old bean (*Phaseolus vulgaris* L. cv. Golden Saxa) plants under non-photorespiratory (2 % O_2) and photorespiratory (21 % O_2) conditions. Low P decreased maximum net photosynthetic rate (P_{Nmax}) and increased the time necessary to reach it. In the leaves of P-deficient plants the relative decrease of P_{Nmax} at 2 % O_2 was larger than at 21 % O_2. The results suggested the influence of photorespiration in the cellular turnover of phosphates.

Additional key words: mannose, net photosynthetic rate, *Phaseolus vulgaris*, photorespiration rate.

Introduction

Phosphorus is the component of several biological compounds. It participates in energy conversion and in transfer of genetic information. Inorganic orthophosphate (P_i) regulates activity of enzymes and metabolic pathways as well as the transport processes involved in the cellular and the whole plant homeostasis (Mimura 1995, Dietz and Harris 1997, Rao 1997, Raghothama 2000, Abel *et al.* 2002).

P_i incorporated into organic phosphates is regenerated during formation of the phosphorus-free final metabolic products. Thus, factors accelerating the rate of metabolic turnover of phosphates may increase the intracellular availability of P_i. A hypothesis was put forward that photorespiration plays a role in the cellular recycling of P_i (Usuda and Edwards 1982, Harris *et al.* 1983, Ray and Portis 1984, Leegood and Furbank 1986, Heber *et al.* 1989) and that its role is enhanced in phosphate-deficient plants (Kozłowska and Maleszewski 1994, Kondracka and Rychter 1997, Kozłowska-Szerenos *et al.* 2000).

In the the present investigation we have analysed the responses of CO_2 exchange in the leaves of orthophosphate well suplied and of orthophosphate deficient bean plants (grown on P_i deficient medium or mannose treated) to 2 % O_2 in atmosphere. The aim of work was to examine how the elimination of photorespiration modifies the effects of phosphorus deficiency.

Materials and methods

French bean (*Phaseolus vulgaris* L. cv. Golden Saxa) plants were cultivated for 2 weeks, in Knop nutrient medium, complete (control) or P_i deficient (-P), in growth chamber, as described previously (Hauschild *et al.* 1996). The nutrient medium, pH 5.7, was continuously aerated and changed every 4 d. Photosynthetic photon flux density (PPFD) at the leaf level was 150 μmol(photons) m^{-2} s^{-1}. Photoperiod was 16 h, day/night temperature 23/19 °C and air humidity about 60 %.

To decrease the content of P_i in the leaves,

Received 14 July 2003, *accepted* 21 March 2004.

Abbreviations: Γ - CO_2 compensation concentration; -P - plants grown on the phosphate-deficient medium; P_i - inorganic orthophosphate; P_N - net photosynthetic rate; P_{Nmax} - maximum net photosynthetic rate; R_D - dark respiration rate; T_{PNmax} - light period necessary to attain P_{Nmax}.

* Corresponding author; fax: (+48) 85 7457302, e-mail: stanmale@uwb.edu.pl

D-mannose, P_i-sequestering factor, was also added in the transpiration stream to the shoots of control plants excited from roots. Mannose solutions 10 mM, 20 mM or 50 mM were used and the treatment was initiated at the zero time of P_N measurements.

Soluble organic and inorganic orthophosphates were assayed by the Fiske-Subbarow method (Fiske and Subbarow 1925) after homogenization and extraction of the primary leaves in cold 10 % TCA. Chlorophylls were extracted with 80 % acetone and assayed spectrophotometrically (*CE 2501*, *CECIL Instruments*, Cambridge, England) according to Lichtenthaler and Wellburn (1983). Glucose was determined using Somogyi-Nelson method (Nelson 1944). Sucrose was determined by the phenol-sulfuric acid method of Dubois *et al*. (1956).

For CO_2 exchange measurements, two attached primary leaves were enclosed into the thermostated (25 ± 0.5 °C) leaf chamber (volume 580 cm^3), included into the closed gas exchange system of the total volume 1025 cm^3, with infrared CO_2/H_2O analyzer (*LI-6262*, *LI-COR*, Logan, USA). The internal atmosphere of the gas system contained 2 % or 21 % O_2, air humidity was close to 100 % and the gas circulation rate 18.6 $cm^3\ s^{-1}$. Photosynthetic photon flux density was 300 μmol $m^{-2}\ s^{-1}$. The CO_2 concentration during measurements of net photosynthetic rate (P_N) varied between 400 to 300 μmol mol^{-1} or dropped to the compensation concentration. Dark respiration (R_D) was measured at the same range of CO_2 concentrations.

Results and discussion

Deficiency of inorganic phosphate in nutrient medium resulted in the significant decrease in content of phosphates in the leaves of experimental plants. The total soluble organic phosphates as well as inorganic orthophosphate (P_i) content in -P plants were approximately 5 times lower in comparison with corresponding controls. The alteration of the other measured parameters of -P leaves (Table 1) presented signs typical of the phosphorus stress: decrease of leaf mass and area and increase of chlorophyll (Chl) and sugar contents.

Table 1. Characteristics of the primary leaves of 2-week-old *Phaseolus vulgaris* L. plants grown on phosphate sufficient (control) or phosphate deficient (-P) medium. For analyses, the leaves were taken from the growth chamber at the middle of light period. Means ± SD (one-side leaf area was considered in all calculations and total phosphates soluble in 10 % trichloroacetic acid).

Parameters	Control	-P	-P/control
Leaf mass [g]	0.81 ± 0.1	0.60 ± 0.1	0.74
Leaf area [cm^2]	44.00 ± 5.0	33.50 ± 6.0	0.76
Chl *a* [mmol m^{-2}]	0.24 ± 0.01	0.34 ± 0.02	1.42
Chl *b* [mmol m^{-2}]	0.10 ± 0.01	0.14 ± 0.01	1.40
P soluble [mmol m^{-2}]	2.81 ± 0.04	0.50 ± 0.01	0.18
P_i [mmol m^{-2}]	1.94 ± 0.03	0.42 ± 0.01	0.22
Sucrose [mmol m^{-2}]	1.11 ± 0.03	1.72 ± 0.04	1.55
Glucose [mmol m^{-2}]	1.27 ± 0.04	2.28 ± 0.05	1.80

To characterize the response of P_N to the depletion of cytosolic P_i, the leaves of control plants were fed with the solution of D-mannose, which sequesters cytosolic P_i (Harris *et al.* 1983). Under 2 % O_2 the progressive fall of P_N during D-mannose feeding was significantly greater than under 21 % O_2 (Fig. 1). This ultimately led to the higher P_N under 21 % O_2 than under 2 % O_2 (Fig. 2). Phosphate deficiency decreased the rate of P_{Nmax} in leaves more under 2 % O_2 than under the 21 % O_2. It also elongated the time (T_{PNmax}) necessary for obtaining P_{Nmax} (Table 2).

The leaf dark respiration rate (R_D) in both groups of experimental plants was dependent on the length of dark period before its measurement. It was the lowest at the end of the night period. The highest R_D was found in leaves of control plants when it was measured directly after reaching P_{Nmax} under 21 % O_2 (Table 3).

The CO_2 compensation concentration of photosynthesis (Γ) was measurable in leaves of both groups of plants only under 21 % O_2. The phosphate deficiency increased always value of Γ, especially in the leaves reaching P_{Nmax} (Table 4).

Cellular P_i plays the key role in regulation of photosynthesis, respiration and the related processes (Raghavendra *et al.* 1994, Dietz and Harris 1997, Wanke *et al.* 1998). Low P_i limits plant metabolism and growth (Fredeen *et al.* 1989, Rao 1997, Ciereszko *et al.* 2002). However, under conditions of insufficient phosphate plant feeding, the rate of P_N was previously found decreased (Fredeen *et al.* 1989, Jacob and Lawlor 1991, 1993, Qiu and Israel 1994), unchanged (Foyer and Spencer 1986, Kondracka and Rychter 1997), or even increased (Ciereszko *et al.* 1996). Divergent changes of R_D, dependent on phosphorus feeding, were also found. Thus the response of P_N and R_D has to depend on additional factors which determine the actual effect of phosphorus deficiency. That may be due to the different efficiency of utilization of endogenous, cytosolic or vacuolar pools of P_i as well as to the modification of alternative metabolic pathways (Mimura 1995, Dietz and Harris 1997, Rao 1997).

In the leaves irradiated under atmospheric O_2 and CO_2 concentrations, photorespiration liberates P_i from phosphorylated intermediates of photosynthetic carbon reduction cycle. Photorespiration increases turnover of phosphates and may be the factor affecting the balance between the organic phosphates and the active pool of P_i within the chloroplast and cytosol (Harris *et al.* 1983, Kozłowska and Maleszewski 1994, Kondracka and Rychter 1997, Kozłowska-Szerenos *et al.* 2000). In addition, it generates several other metabolites (Wingler *et al.* 2000), thus may interact with the photosynthesis and respiration in variety of ways.

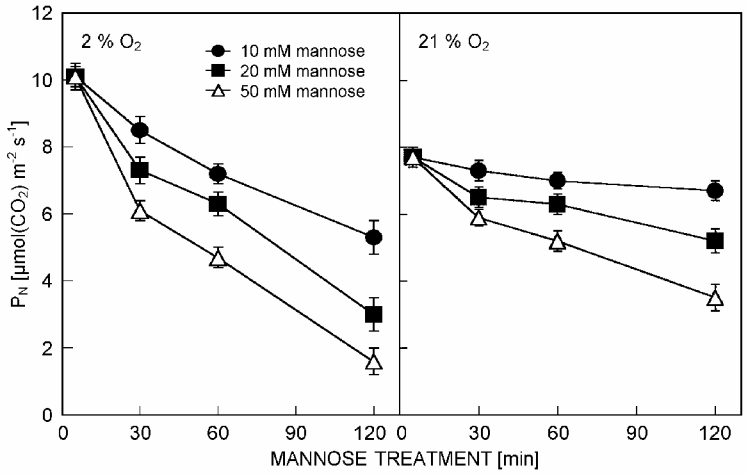

Fig. 1. Responses of net photosynthetic rate (P_N) in primary leaves of bean (*Phaseolus vulgaris* L.) to the depletion of cytosolic phosphate by D-mannose feeding under 2 % and 21 % O_2. In experiments plants grown on phosphate-sufficient nutrient medium (control) were used. During measurements PPFD was 500 μmol m^{-2} s^{-1}, the temperature was 25 ± 0.5 °C. Mean value ± SD.

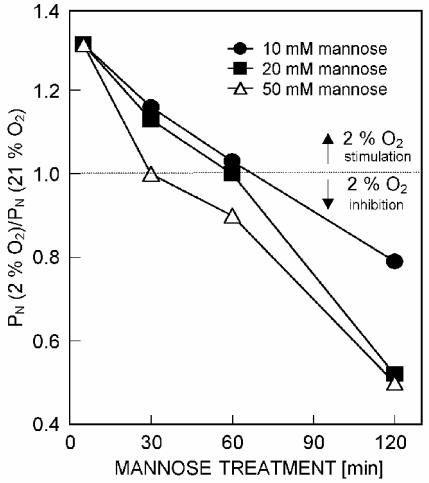

Fig. 2. Changes in P_N 2 % O_2/P_N 21 % O_2 ratio in the primary leaves of bean (*Phaseolus vulgaris* L.) during depletion of cytosolic phosphate by D-mannose feeding. Data calculated on the basis of results presented on Fig. 1.

Results of experiments with plants fed with exogenously supplied mannose, the cytosolic phosphate-sequestering compound (Harris *et al.* 1983) strongly justify the above assumption. In our experiments, effect of mannose strongly depended on O_2 concentration in atmosphere. Under 21 % O_2, the uptake of 10 mM mannose solution practically had no effect on the P_N of the leaves. The 50 mM mannose decreased the P_N by about 50 %, after 2 h uptake (Fig.1). However, when the photorespiration was eliminated under the conditions of 2 % O_2, the effect of mannose was much faster, and 50 mM mannose inhibited the P_N in leaves more than 80 % (Figs.1, 2). In the leaves treated with mannose for 2 h even the higher P_N under 21 % than under 2 % of oxygen (inverse Warburg effect) was observed (Fig. 2). A possibility that mannose feeding have altered P_N also by modifying the stomatal conductance could be considered, but this possibility was not experimentally controlled in the presented investigation.

Table 2. The maximum rate of net photosynthesis (P_{Nmax}) [μmol (CO_2) m^{-2} s^{-1}] and the light period necessary to attain it (T_{PNmax}) [min] in the primary leaves of *Phaseolus vulgaris* control and phosphate deficient (-P) plants, measured under 2 and 21 % O_2. During light activation and measurements of P_N, the PPFD was 300 μmol m^{-2} s^{-1}, the temperature was 25 ± 0.5 °C. Means ± SD.

O_2	Parameters	Control	-P	-P/control
2 %	P_{Nmax}	7.0 ± 0.2	4.8 ± 0.7	0.69
	T_{PNmax}	68.0 ± 3.0	83.0 ± 3.0	1.20
21 %	P_{Nmax}	4.0 ± 0.3	3.5 ± 0.2	0.88
	T_{PNmax}	62.0 ± 4.0	73.0 ± 4.0	1.20

Table 3. The dark respiration rate (R_D) [nmol(CO_2) m^{-2} s^{-1}] in the primary leaves of *Phaseolus vulgaris* control and phosphate deficient (-P) plants, measured under 2 % and 21 % O_2, at the end of the night period (1), after full light activation of P_N (2), and after subsequent 2 h of dark (3). The temperature during measurements was 25 ± 0.5 °C. Means ± SD.

O_2		Control	-P	-P/control
2 %	(1)	0.09 ± 0.01	0.06 ± 0.01	0.7
	(2)	0.16 ± 0.02	0.18 ± 0.03	1.1
	(3)	0.13 ± 0.02	0.12 ± 0.02	0.9
21 %	(1)	0.10 ± 0.02	0.09 ± 0.01	0.9
	(2)	0.26 ± 0.01	0.18 ± 0.01	0.7
	(3)	0.16 ± 0.03	0.16 ± 0.02	1.0

Table 4. The CO_2 compensation concentration of photosynthesis (Γ) [μmol(CO_2) mol^{-1}] in the primary leaves of *Phaseolus vulgaris* control and phosphate deficient (-P) plants measured under 21 % O_2, at the beginning of light period (1), and after full light activation of P_N (2). During measurements PPFD was 300 μmol m^{-2} s^{-1}, the temperature was 25 ± 0.5 °C. Means ± SD.

	Control	-P	-P/control
(1)	21 ± 1	25 ± 1	1.2
(2)	22 ± 1	31 ± 5	1.4

The decrease of P_{Nmax} in -P leaves was significantly lower at 21 % than at 2 % O_2 concentration (Table 2). Undoubtedly, this was the result of the two contrary effects of 21 % oxygen. At atmospheric concentration O_2 increases the oxygenase activity of Rubisco and thus reduces P_N. Simultaneously, it may stimulate P_N due to photorespiratory enhancement of phosphates turnover rate. Small effect of phosphate deficiency on P_{Nmax} under 21 % O_2 and the declined capacity of 2 % O_2 to stimulate P_{Nmax}, found in our experiments (Table 2), also allow to suggest that photorespiration may restrict the effects of insufficient phosphorus feeding. Increased content of free sucrose and glucose (Table 1), as well as the higher Γ, are the additional confirmations of enhanced P turnover in the -P leaves under the conditions of 21 % O_2.

Phosphate deficiency significantly increased T_{PNmax}. The extension of T_{PNmax} did not depend on the concentration of O_2 (Table 2). This can be explained by the fact that leaf P_i has only small effect on the photosynthetic light reactions (Abadia *et al.* 1987) and electron transport (Jacob and Lawlor 1993). Whereas, the light activation of photosynthesis depends mainly on the building up the pools of the phosphorylated intermediates of the photosynthetic carbon reduction cycle.

At the end of the night period in control plants, no effect of 2 % O_2 on R_D was found. Whereas, in the leaves of -P plants the R_D rate at 2 % O_2 was significantly lower than at 21 % O_2 (Table 3). These findings suggest that phosphate deficiency may affect the dependence of R_D on the O_2 concentration.

A long period of irradiation stimulates the rate of leaf respiration, probably due mainly to accumulation of sugars (Azcon-Bieto 1992, Raghavendra *et al.* 1994). In our investigation full light activation of photosynthesis also significantly increased the rate of R_D in leaves control and -P plants (Table 3). However, the highest rate of R_D was reached in control plants at 21 % O_2. No effect of 21 % O_2 on R_D in leaves of -P plants with fully activated P_N was probably due to the low content of soluble sugar phosphates. The results suggest that the modifications of cell phosphate homeostasis dependent on photorespiration may also affect the respiratory metabolism.

References

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

Abel, S., Ticconi, C.A., Delatorre, C.A.: Phosphate sensing in higher plants. - Physiol. Plant. **115**: 1-8, 2002.

Azcon-Bieto, J.: Relationships between photosynthesis and respiration in the dark in plants. - In: Barber, J., Guerrero, M.G., Medrano, H. (ed.): Trends in Photosynthesis Research. Pp. 241-253. Intercept, Andover 1992.

Ciereszko, I., Gniazdowska, A., Mikulska, M., Rychter, A.M.: Assimilate translocation in bean (*Phaseolus vulgaris* L.) during phosphate deficiency. - J. Plant Physiol. **149**: 343-348, 1996.

Ciereszko, I., Janonis, A., Kociakowska, M.: Growth and metabolism of cucumber in phosphate-deficient conditions. - J. Plant Nutr. **25**: 1115-1127, 2002.

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

Dubois, M., Gilles, K.A., Hamilton, J.K., Rebus, P.A., Smith, F.: Colorimetric method for the determination of sugars and related substances. - Anal. Chem. **28**: 350-356, 1956.

Fiske, C.H., Subbarow, Y.: The colorimetric determination of phosphorus. - J. biol. Chem. **66**: 375-400, 1925.

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

Fredeen, A.L., Rao, I.M., Terry, N.: Influence of phosphate nutrition on growth and carbon partitioning in *Glycine max*. - Plant Physiol. **89**: 225-230, 1989.

Harris, G.C., Cheesbrough, J.K., Walker, D.: Effects of mannose on photosynthetic gas exchange in spinach leaf

discs. - Plant Physiol. **71**: 108-111, 1983.

Hauschild, T., Ciereszko, I., Maleszewski, S.: Influence of phosphorus deficiency on post-irradiation burst of CO_2 from bean (*Phaseolus vulgaris* L.) leaves. - Photosynthetica **31**: 1-9, 1996.

Heber, U., Vill, J., Nemanis, S., Mimura, T., Dietz, K.J.: Photoinhibitory damage to chloroplasts under phosphate deficiency and alleviation of deficiency and damage by photorespiratory reactions. - Z. Naturforsch. **44**: 524-536, 1989.

Jacob, J., Lawlor, D.W.: Stomatal and mesophyll limitations of photosynthesis in phosphate deficient sunflower, maize and wheat plants. - J. exp. Bot. **42**: 1003-1011, 1991.

Jacob, J., Lawlor, D.W.: *In vivo* photosynthetic electron transport does not limit photosynthetic capacity in phosphate-deficient sunflower and maize leaves. - Plant Cell Environ. **16**: 785-795, 1993.

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

Kozłowska, B., Maleszewski, S.: Low level of inorganic orthophosphate in growth medium increases metabolism and excretion of glycolate by *Chlorella vulgaris* cells cultivated under air conditions. - Plant Physiol. Biochem. **32**: 717-721, 1994.

Kozłowska-Szerenos, B., Zielinski, P., Maleszewski, S.: Involvement of glycolate metabolism in acclimation of *Chlorella vulgaris* cultures to low phosphate supply. - Plant Physiol. Biochem. **38**: 727-734, 2000.

Leegood, R.C., Furbank, R.T.: Stimulation of photosynthesis by 2 % oxygen at low temperatures is restored by phosphate. - Planta **168**: 84-93, 1986.

Lichtenthaler, H.K., Wellburn, A.R.: Determination of total carotenoids and chlorophylls *a* and *b* of leaf extracts in different solvents. - Biochem. Soc. Trans. **603**: 591-592, 1983.

Mimura, T.: Homeostasis and transport of inorganic phosphate in plants. - Plant Cell Physiol. **36**: 1-7, 1995.

Nelson, N.A: Photometric adaptation of the Somogyi method for determination of glucose. - J. biol. Chem. **153**: 375-380, 1944.

Qiu, I., Israel, D.W.: Carbohydrate accumulation and utilization in soybean plants in response to altered phosphorus nutrition. - Physiol. Plant. **90**: 722-728, 1994.

Raghavendra, A.S., Padmasree, K., Saradadevi, K.: Interdependence of photosynthesis and respiration in plant cells: interactions between chloroplasts and mitochondria. - Plant Sci. **97**: 1-14, 1994.

Raghothama, K.G.: Phosphate transport and signaling. - Curr. Opinion Plant Biol. **3**: 182-187, 2000.

Rao, I.M.: The role of phosphorus in photosynthesis. - In: Pessarakli, M. (ed.): Handbook of Photosynthesis. Pp. 173-194. Marcel Dekker, New York 1997.

Ray, P.G., Portis, A.R., Jr.: Regulation of photosynthetic carbon metabolism under photorespiratory and non-photorespiratory conditions. - In: Sybesma, C. (ed.): Advances in Photosynthesis Research. Vol. 9. Pp. 821-824. Kluwer Academic Publishers, Dordrecht 1984.

Usuda, H., Edwards, G.E.: Influence of varying CO_2 and orthophosphate concentration on rates of photosynthesis, and synthesis of glycolate and dihydroxyacetone phosphate by wheat chloroplasts. - Plant Physiol. **69**: 469-473, 1982.

Wanke, M., Ciereszko, I., Podbielkowska, M., Rychter, A.M.: Response to phosphate deficiency in bean (*Phaseolus vulgaris* L.) roots. Respiratory metabolism, sugar localization and changes in ultrastructure of bean root cells. - Ann. Bot. **82**: 809-819, 1998.

Wingler, A., Lea, P.J., Quick, P., Leegood, R.: Photorespiration: metabolic pathways and their role in stress protection. - Phil. Trans. roy. Soc. London B **355**: 1517-1529, 2000.