BIOLOGIA PLANTARUM 41 (2): 217-226, 1998

# Growth, nitrate uptake and respiration rate in bean roots under phosphate deficiency

A. GNIAZDOWSKA, M. MIKULSKA and A.M. RYCHTER

*Institute of Experimental Plant Biology, University of Warsaw, Pawińskiego 5A, 02-106 Warszawa, Poland*

## Abstract

The decrease in inorganic phosphate concentration in bean (*Phaseolus vulgaris* L. cv. Złota Saxa) roots induced decrease in respiration rate. The decrease observed in ATP pool in phosphate deficient (-P) roots was greater than it would result from the decline in respiration and possible involvement of alternative pathway, suggesting an increased energy utilization for growth and ion uptake. Indeed, relative growth rate was higher in -P plants until 12 d of culture and later dropped to the rate similar to the control. Net nitrate uptake rate was higher in -P plants than in +P plants at the beginning of phosphate starvation, then during the prolonged culture it decreased rapidly in -P plants and after 19 d it was 8 times lower than that in the control. The decline in ATP production during prolonged phosphate starvation influenced $NO_3^-$ uptake more than root growth.

*Additional key words*: ATP, cyanide resistant respiration, *Phaseolus vulgaris*.

## Introduction

In the roots, growth, maintenance and ion uptake are three major energy-requiring processes in which respiratory energy is utilised. Anion uptake can be assessed as judged from nitrate uptake as its energy cost is the largest. It is assumed that little respiratory energy is involved in the uptake of cations (Van der Werf *et al.* 1988). The energy involved in ion uptake depends on the number of active root-membrane passages per ion and the proton-ion and proton-ATP stoichiometries (Bouma *et al.* 1995).

---

*Received* 16 December 1997, *accepted* 1 April 1998.

*Abbreviations*: DCCD - N,N'-dicyclohexylcarbodiimide; DES - dietylstilbestrol; DMSO - dimethyl sulfoxide; NNUR - net nitrate uptake rate; PCA - perchloric acid; RGR - relative growth rate; SHAM - salicylhydroxamic acid; TCA - trichloro-aceticic acid.

*Acknowledgements*: We would like to thank Mrs. Elżbieta Sakławska for skilful technical assistance. This work was in part supported by Grant no 6P204 037 05 and Grant No. 6PO4C 03413 given to A.R. from the State Committee for Scientific Research (Poland).

Fax: (+48) 22 6584804, e-mail: gniazdowska@ibbrain.ibb.waw.pl

Decrease in inorganic phosphate concentration affects tissue respiration activating the alternative pathway and thereby influences the ATP content in cells (Rychter and Mikulska 1990, Rychter *et al.* 1992). On the other hand, one of the most pronounced effects of phosphate starvation is the increase in fresh and dry masses of roots (Rychter and Mikulska 1990, Rao *et al.* 1993, Ciereszko *et al.* 1996, Kondracka and Rychter 1997) but uptake and assimilation of $NO_3^-$ calculated per root mass or root surface area unit is lower in phosphate deficient plants (Rufty *et al.* 1990, 1993).

Growth and respiration are integrated by the sharing of common carbohydrate and adenylate components (de Visser 1987), while ion uptake, assimilation and respiration are integrated by electron transfer and ATP consumption and production, respectively. The energy that drives nitrate uptake derives from the proton gradient maintained across the plasma membrane by $H^+$-ATPase (Crawford 1995); uptake of 1 mole of nitrate consumes 1 to 2 moles of ATP (Imsande and Touraine 1994).

The aim of paper was to examine the relationship between ATP concentration, respiration rate, growth rate and nitrate uptake rate in roots of bean plants grown on phosphate deficient medium.

## Materials and methods

**Plants:** *Phaseolus vulgaris* L. cv. Złota Saxa were grown on 3 $dm^3$ containers (6 plants per pot) filled with either a complete or phosphate-deficient Knop nutrient solution (Rychter and Mikulska 1990). The culture medium was continuously aerated and changed every 3 d. The cotyledons were removed when seedlings were 8 d old. Plants were grown in a growth chamber under 16-h photoperiod and photon flux density of 150 $\mu mol\ m^{-2}\ s^{-1}$. Day/night temperatures were 24/20 °C and air humidity was 80 %. Plants at the age of 8, 11, 13, 15 or 19 d were taken for determinations. The period of phosphate starvation was 4 d less than the age of plants.

**Root respiration:** Oxygen uptake by the roots of intact plants was measured polarographically at 20 °C, using a Clark oxygen electrode (*YSI*, USA). Roots (1 - 3 depending on the size) were sealed into the 90-$cm^3$ cuvette containing a nutrient solution identical to that for growing of the plants and was air-saturated before the start of the measurements. Salicylhydroxamic acid (SHAM), dissolved in dimethyl sulfoxide (DMSO), and KCN, in distilled water (pH was adjusted to 7.0) were added directly to the cuvette at a final concentration 1.7 and 1 mM, respectively. When the effect of SHAM was examined, the next reading took place after a 60-min preincubation of the roots with the inhibitor. The appropriate concentration of each inhibitor was estimated from titration curves.

**Inorganic phosphate** was determined in dry material after homogenization and extraction with 10 % trichloro-acetic acid (TCA) using method of Fiske and Subbarow (Rychter and Mikulska 1990).

**ATP level** was measured in the whole root system of a single plant. Roots were rapidly blotted with filter paper, weighed and ground in a cool mortar containing 1 $cm^3$ of 10 % perchloric acid (PCA) per 1 g tissue, to which 1 $mm^3$ of concentrated PCA was added per 10 mg of fresh root mass (Rychter *et al.* 1992). The ground material was centrifuged at 5 000 *g* for 5 min. The PCA was then removed from supernatant by partitioning in a trioctylamine/freon solution. After centrifugation the top layer, consisting of the neutralized extract, was decanted and stored at -20 °C. ATP was determined using the luciferin-luciferase reaction according to Pradet (1967).

**Tissue analyses:** Six randomly chosen plants of ages 8, 11, 13, 15 or 19 d were harvested, separated into shoot (leaves and stem) and roots, blotted with filter paper, and weighed. Dry mass was determined after drying the plant tissues for 48 h at 80 °C. Relative growth rate (RGR) was determined as the slope of the natural logarithm of plant dry mass against time (Van der Werf *et al.* 1988). Total nitrogen was determined in dried plant material using a modified Kjeldahl method (Van der Werf *et al.* 1988). The N content was determined colorimetrically by autoanalyser. The net rate of nitrogen uptake per unit root mass (NNUR) was calculated from the RGR, the total plant organic plus inorganic nitrogen concentration, and the relative amount of root biomass (Van der Werf *et al.* 1988). Nitrate uptake was determined by measuring its disappearance from the nutrient solution at 3-d intervals. Nitrate ($NO_3^-$) content in the medium was determined colorimetrically after reduction to nitrite by addition of 5 % salicylic acid in sulphuric acid and 2 M NaOH (Cataldo *et al.* 1975).

**Statistics:** Each value shown in the figures and tables is the average ± SD of 6 determinations performed on plants grown in at least 3 independent experiments. The RGR and NNUR functions were approximated using an *Easy Plot Computer Program.*

## Results

Inorganic phosphate (Pi) concentration in roots and shoots of control (+P) plants growing on adequate phosphate supply remained constant during the experiment (Table 1). In shoots of 8-d-old plants growing on phosphate deficient medium (-P) the phosphate concentrations were similar to the control, but decreased during the prolonged culture. In the shoots of 19-d-old plants Pi content deceased to about 13 % of that in control plants. In the roots of -P plants the phosphate concentration was noticeably lower just at the beginning of the culture and after 15 d of culture on phosphate deficient medium declined to about 6 % of that of control (Table 1).

The rates of root respiration of +P and -P plants were similar at the beginning of the culture but after longer period decreased more in -P than +P roots, in 19-d-old -P roots the rates of $O_2$ uptake were about 60 % of the control (Fig. 1).

Table 1. Inorganic phosphate concentration [mg $g^{-1}$(d.m.)] and shoot and root dry mass [g $plant^{-1}$] of plants cultured on phosphate sufficient (+P) or phosphate deficient (-P) medium. Means ± SD; $n = 9$.

| Plant part | Plant age | Phosphate | | Dry mass | |
|---|---|---|---|---|---|
| | [d] | +P | -P | +P | -P |
| Shoot | 8 | 20.21 ± 2.11 | 22.91 ± 1.47 | 0.06 ± 0.01 | 0.05 ± 0.01 |
| | 11 | 29.73 ± 4.62 | 9.04 ± 1.61 | 0.22 ± 0.02 | 0.20 ± 0.02 |
| | 13 | 16.66 ± 2.44 | 5.30 ± 0.37 | 0.28 ± 0.02 | 0.26 ± 0.01 |
| | 15 | 19.72 ± 2.99 | 4.46 ± 1.46 | 0.66 ± 0.07 | 0.50 ± 0.08 |
| | 19 | 18.01 ± 1.71 | 2.40 ± 0.31 | 1.03 ± 0.13 | 0.52 ± 0.05 |
| Root | 8 | 18.00 ± 1.16 | 7.22 ± 1.14 | 0.01 ± 0.00 | 0.01 ± 0.00 |
| | 11 | 18.50 ± 3.86 | 3.37 ± 1.24 | 0.06 ± 0.01 | 0.07 ± 0.02 |
| | 13 | 19.19 ± 3.44 | 2.52 ± 0.47 | 0.07 ± 0.01 | 0.09 ± 0.01 |
| | 15 | 20.36 ± 3.46 | 2.08 ± 0.87 | 0.15 ± 0.03 | 0.18 ± 0.04 |
| | 19 | 20.12 ± 3.62 | 1.23 ± 0.20 | 0.20 ± 0.05 | 0.22 ± 0.06 |

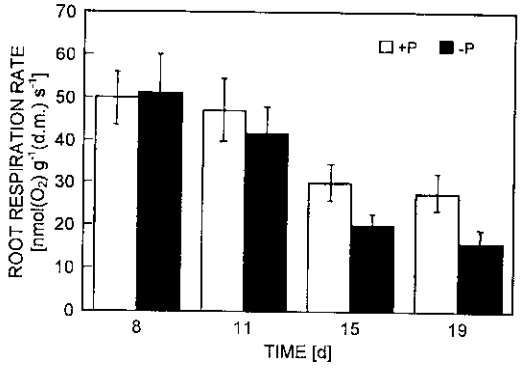


Fig. 1. Root respiration rates of bean plants cultured on phosphate sufficient (+P) or phosphate deficient (-P) medium. Means ± SD, $n = 5$.

Oxygen uptake of roots of +P and -P plants after 11 d of culture was inhibited 30 - 40 % by KCN. This inhibition was similar during the whole cultivation period of +P plants. In -P roots the inhibition of respiration by KCN decreased with prolonged culture on phosphate deficient medium, in 15- and 19-d-old plants was only 18 and 12 %, respectively (Fig. 2). The remaining, cyanide insensitive respiration was inhibited by addition of SHAM (results not shown).

Oxygen uptake was also measured at various concentrations of SHAM in absence of KCN. No stimulation of $O_2$ uptake was observed in the presence of low concentration (1.7 - 3.5 mM) of SHAM, indicating no induction of $O_2$-consuming peroxidase activity (data not shown). SHAM reduced the total respiration of 11-d-old +P and -P roots by 10 and 20 %, respectively. In 16-d-old -P plants SHAM reduced root respiration by 30 % (Fig. 2). In plants cultured on phosphate deficient medium

the increase in sensitivity of respiration to SHAM was accompanied by the decrease in its sensitivity to KCN, whereas in phosphate sufficient roots the level of inhibition by both KCN and SHAM was similar during entire culture period. These results may indicate the increased participation of the alternative pathway in respiration of -P roots as compared to the control, and therefore a decreased rate of ATP production by mitochondrial respiratory chain.

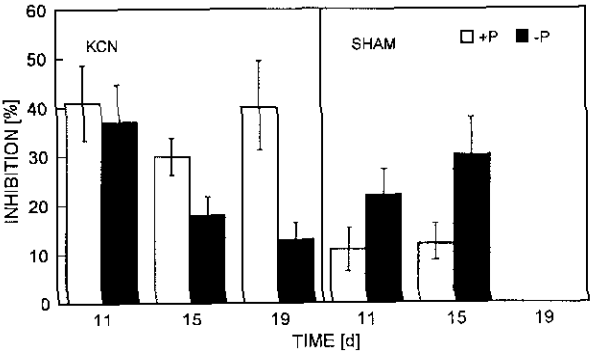


Fig. 2. KCN inhibition and SHAM inhibition of root respiration of bean plants cultured on phosphate sufficient (+P) or phosphate deficient (-P) medium. Means ± SD, $n = 5$.

The ATP concentration in roots of beans grown on complete nutrient medium decreased slightly during the culture (Fig. 3). In the roots of 8-d-old -P plants ATP level was only 13 % lover than in +P roots. The prolonged phosphate starvation resulted in the further decrease in ATP concentration, in roots of 15-d-old plants ATP content was 42 % of the control and in 19-d-old 28 % of the control only (Fig. 3).

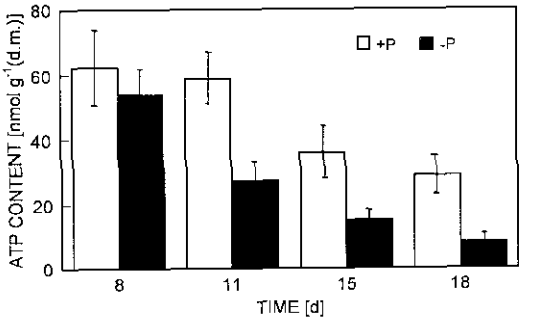


Fig. 3. ATP content in bean roots during the culture of plants on phosphate sufficient (+P) or phosphate deficient (-P) medium. Means ± SD, $n = 9$.

Plant culture on -P nutrient medium resulted in a distinct alterations in growth rate. No differences in root and shoot dry masses were observed at the beginning of the culture up to day 13. However, dry masses of stem and leaves of 15 and 19-d-old

-P plants were lower than those of the control, while root dry mass accumulation was similar or even slightly greater than in +P plants (Table 1). The ratio of dry mass/fresh mass did not change with plant age; it was 0.17 and 0.16 in +P and -P plants, respectively (results not shown).

In 8-d-old +P and -P bean plants the ATP content was similar (18 nmol $root^{-1}$) (data from Table 1 and Fig. 3). In control plants the ATP content increased in parallel with the increase in root mass and reached about 62 nmol $root^{-1}$ in 19-d-old plants, while in roots of phosphate deficient plants it reminded at constant level, about 20 nmol $root^{-1}$ independently of root mass.

Dynamics of the increase of root dry mass of +P and -P plants were different (Fig. 4). The RGR in -P plants was higher than in +P plants at the beginning of the experiment. During the next 5 d (between day 11 to 15 of culture) the RGR of +P and -P roots decreased to the same level. After 16 d it was insignificantly lower in -P plants.

Nitrate uptake from the nutrient solution (measured as disappearance of $NO_3$ from culture medium) was constant during the culture for -P plants 16 [mg($NO_3$) $plant^{-1}$ $d^{-1}$], while for control plants it increased during growing period from 18 to 40 [mg($NO_3$) $plant^{-1}$ $d^{-1}$].

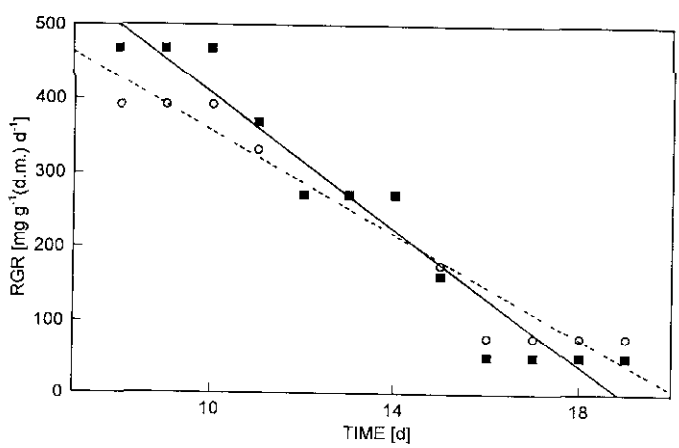


Fig. 4. Root relative growth rate of bean plants cultured on phosphate sufficient (+P) or phosphate deficient (-P) medium. RGR was determined as the slope of the natural logarithm of plant dry mass versus time (+P - *open circles*, -P - *closed squares*). The RGR function of +P: y = -46x + 866 (*dashed line*) and of -P: y = -35x + 708 (*full line*) were approximated using an *Easy Plot Computer Program*.

In control plants total nitrogen content increased during culture period. Up to 15 d of culture -P plants accumulated the same amount of N as +P plants (Fig. 5). The decrease in N accumulation in -P plants was evident by day 15. Until the end of experiment N content in -P plants remained constant and in 19-d-old -P beans was half of the control.

The net nitrate uptake rate (NNUR) can be considered as rate of ion uptake by the root. NNUR is calculated as an increase of N-content of the whole plant per root

mass as function of time. For control roots NNUR showed slight variations from 2.94 to 1.84 during the cultivation period (Fig. 6). The NNUR of -P plants decreased from

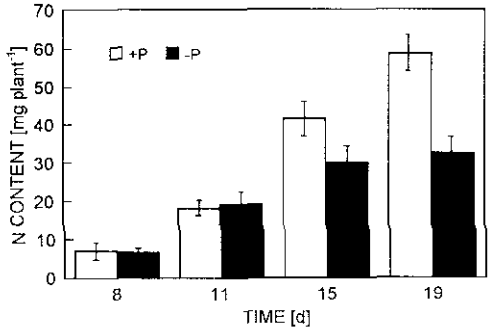


Fig. 5. Total N accumulation by bean plants cultured on phosphate sufficient (+P) or phosphate deficient (-P) medium. Means ± SD, $n = 9$.

3.64 at the beginning of the culture to 0.24 after two weeks of phosphate starvation (Fig. 6). The decrease of NNUR of -P plants until day 15 was similar to the decrease of the RGR of -P plants (Fig. 4 and Fig. 6) The prolonged phosphate starvation resulted in a rapid decrease of NNUR for -P plants, and at the end of culture the rate of NNUR for -P plants was 7.6 times lower than for +P plants (Fig. 6).

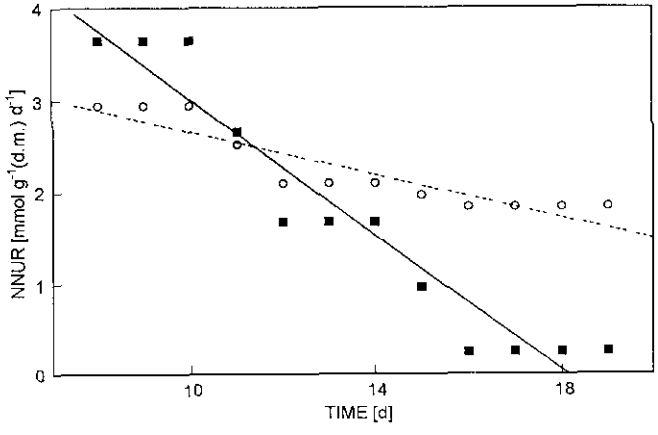


Fig. 6. The net rate of nitrate uptake of bean plants cultured on phosphate sufficient (+P) or phosphate deficient (-P) medium. NNUR was calculated from the RGR, the total plant nitrogen concentration, and the relative amount of root biomass (+P - *open circles*, -P - *closed squares*). The NNUR function of +P: y = - 0.117x + 3.83 (*dashed line*) and of -P: y = - 0.371x + 6.72 (*full line*) were approximated using an *Easy Plot Computer Program*.

## Discussion

ATP concentration decreased rapidly in the roots of -P plants; after 11 d of culture (7 d on -P medium) it was about half that of the control and dropped to one-third of

the control in 19-d-old plants (Fig. 3). The cell ATP level reflects both the rate of its synthesis and utilisation, while the rate of ATP synthesis is highly controlled by ATP demand. In -P plants, despite of the high demand due to the increased growth rate, ATP synthesis may be lower because of decrease of Pi availability for oxidative phosphorylation and the participation of alternative pathway of respiration.

In order to determine the alternative pathway engagement in total respiration, the ratio SHAM inhibition to KCN resistance has been calculated. However, the results of Hoefnagel *et al.* (1995) indicated that electrons can be diverted from the alternative pathway to the cytochrome pathway during SHAM inhibition. It means that cytochrome pathway does not have to be saturated for the alternative pathway to be operating (Atkin *et al.* 1995) and the degree of SHAM inhibition may underestimate the participation of alternative pathway in total respiration. Therefore it is impossible to determine the exact involvement of alternative pathway and thus to calculate ATP production (Day *et al.* 1996). According to that, we can only say that in our experimental conditions in 16-d-old plants the ratio SHAM inhibition/KCN resistance of respiration was higher for -P (0.37) than for +P roots (0.17) suggesting the greater involvement of the alternative pathway in root respiration of -P plants.

Although in -P plants the decrease in ATP content (Fig. 3) was correlated with a decline in total respiration rate (Fig. 1) and increase of KCN resistance (Fig. 2), the decrease in ATP content was greater than the decline in respiration and possible increase of alternative pathway activity. This may indicate the greater ATP utilisation in -P roots.

Root growth rate (RGR) was higher in 8- to 10-d-old -P bean plants than in the control (Fig. 4) suggesting that in -P young plants ATP consumption for growth increased as compared to the control. While ATP content decreased rapidly at day 15, the RGR of -P roots declined and was similar to the control. It could be concluded that after 2 weeks of phosphate starvation ATP may be limiting for biomass production and the increase in root mass of -P plants (Table 1) was the result of higher RGR at the beginning of the culture. The decreased root growth of -P plants was observed despite higher C-export from the leaves to the roots (Ciereszko *et al.* 1996). Higher glucose and fructose content found in the roots of phosphate-deficient beans seemed to represent non-phosphorylated, probably non-metabolic pool, which could not be utilized for growth (Rychter and Randall 1994).

Rufty *et al.* (1993) suggested that during phosphate starvation low ATP might restrict active $NO_3^-$ influx against an electrochemical potential gradient or limit synthesis of the membrane transport system for $NO_3^-$. The absorption of nitrate by roots of higher plants is active and require an input of energy (Warner and Kleinhofs 1992, Crawford 1995). $NO_3^-$ transport system is linked to the operation of the plasmalemma $H^+$-ATPase, and the link between $NO_3^-$ absorption and the $H^+$-ATPase appears to be due to a $H^+$ - $NO_3^-$ symport which has flux stoichiometry >1, (MacClure *et al.* 1990a,b). The activity of plasma membrane proton pump can directly influence $NO_3^-$ uptake (Santi *et al.* 1995). The inhibitors of plasmalemma ATPase (DCCD, orthovanadate, and DES) decrease nitrate uptake (Kłobus 1990).

In our experiment, we measured $NO_3^-$ uptake as its disappearance from nutrient solution which is only an approximate measurement of $NO_3^-$ uptake. According to

these determinations $NO_3^-$ uptake from nutrient medium by -P bean roots was independent from increase of absorbing area of the root; $NO_3^-$ uptake per plant was constant during experiment, while in control plants ion uptake increased with root mass. $NO_3^-$ uptake from culture medium was correlated with total N content in plants (Fig. 5) and net nitrate uptake rate (NNUR) (Fig. 6).

The stimulation of root growth (RGR) of -P plants at the beginning of the culture was accompanied by high level of NNUR, both of those processes contributed to the decrease in ATP pool earlier and to a greater extent than it would result from the decrease of respiration.

Experiments in which both nitrate availability and plant growth rate were manipulated independently showed that nitrate uptake rates are determined mainly by regulatory processes that co-ordinate nitrate uptake and biomass production (Imsande and Touraine 1994). Therefore inhibition of biomass production would greatly influence nitrate uptake rate. In our studies we indicated that inhibition of NNUR during phosphate deficiency occurs prior to the inhibition of RGR.

The decrease in Pi in the tissue changed the energy economy of the root. As the result of phosphate deficiency lower respiration rate and possible involvement of alternative pathway decreased the rate of ATP synthesis despite its higher demand for growth. However, at the beginning of phosphate starvation, stimulation of growth and higher NNUR was observed. ATP utilisation mainly contributed to significant decrease in ATP level in 11-d-old plants. After 15 d of culture lower ATP production during respiration also contributed to the decrease in ATP concentration. Low ATP level at this period affected more net nitrate uptake rate than growth rate. It means that during phosphate deficiency, in limited ATP pool, maintaining of root growth process necessary for phosphate acquisition from outside is the most important and represent a strategy for plant survival.

## References

Atkin, O.K., Villar, R., Lambers, H.: Partitioning of electrons between the cytochrome and alternative pathways in intact roots. - Plant Physiol. **108**: 1179-1183, 1995.

Bouma, T.J., Broekhuysen, A.G.M., Veen, B.W.: Utilization of Respiratory Energy in Higher Plants. Requirements for "Maintenance" and Transport Processes. - Thesis. Landbouw-Universiteit, Wageningen 1995.

Cataldo, D.A., Haroon, M., Schrader, L.E., Youngs, V.L.: Rapid colorimetric determination of nitrate in plant tissue by nitration of salicylic acid. - Commun. Soil Sci. Plant Anal. **6**: 71-80, 1975.

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

Crawford, N.M.: Nitrate: nutrient and signal for plant growth. - Plant Cell **7**: 859-868, 1995.

Day, D.A., Krab, K., Lambers, H., Moore, A.L., Siedow, J.N., Wagner, A.M., Wiskich, J.T.: The cyanide-resistant oxidase: to inhibit or not to inhibit, that is the question. - Plant Physiol. **110**: 1-2, 1996.

de Visser, R.: On the integration on plant growth and respiration. - In: Moore, A.L., Beechey, R.B. (ed.): Plant Mitochondria. Structural, Functional and Physiological Aspect. Pp. 331-340. Plenum Press, New York 1987.

Hoefnagel, M.H.N., Millar, A.H., Wiskich, J.T., Day, D.A.: Cytochrome and alternative respiratory pathways compete for electrons in the presence of pyruvate in soybean mitochondria. - Arch. Biochem. Biophys. **318**: 394-400, 1995.

Imsande, J., Touraine, B.: N demand and the regulation of nitrate uptake. - Plant Physiol. **105**: 3-7, 1994.

Kłobus, G.: Nitrate uptake and activity of plasmalemma associated ATPase in *Cucumis sativus* L. roots. - Acta Physiol. Plant. **12**: 225-231, 1990.

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

McClure, P.R., Kochian, L.V., Spanswick, R.M., Shaff, J.E.: Evidence for cotransport of nitrate and protons in maize roots. I. Effect of nitrate on membrane potential. - Plant Physiol. **93**: 281-289, 1990a.

McClure, P.R., Kochian, L.V., Spanswick, R.M., Shaff, J.E.: Evidence for cotransport of nitrate and protons in maize roots. II. Measurement of $NO_3^-$ and $H^+$ fluxes with ion-selective microelectrodes. - Plant Physiol. **93**: 290-294, 1990b.

Pradet, A.: Étude des adénosine-5-mono-, di-, et tri-phosphate dans les tissues végétaux. I. Dosage enzymatique. - Physiol. vég. **5**: 209-221, 1967.

Rao, I.M., Fredeen, A.L., Terry, N.: Influence of phosphorus limitation on photosynthesis, carbon allocation and partitioning in sugar beet and soybean grown with a short photoperiod. - Plant Physiol. Biochem. **31**: 223-231, 1993.

Rufty, T.W., Jr., MacKown, C.T., Israel, D.W.: Phosphorus stress effects on assimilation of nitrate. - Plant Physiol. **94**: 328-333, 1990.

Rufty, T.W., Jr., Israel, D.W., Volk, R.J., Qiu, J., Sa, T.: Phosphate regulation of nitrate assimilation in soybean. - J. exp. Bot. **44**: 879-891, 1993.

Rychter, A.M., Mikulska, M.: The relationship between phosphate status and cyanide-resistant respiration in bean roots. - Physiol. Plant. **79**: 663-667, 1990.

Rychter, A.M., Chauveau, M., Bomsel, J-L., Lance, C.: The effect of phosphate deficiency on mitochondrial activity and adenylate levels in bean roots. - Physiol. Plant. **84**: 80-86, 1992.

Rychter, A.M., Randall, D.D.: The effect of phosphate deficiency on carbohydrate metabolism in bean roots. - Physiol. Plant. **91**: 383-388, 1994.

Santi, S., Locci, G., Pinton, R., Cesco, S., Varanini, Z.: Plasma membrane $H^+$-ATPase in maize roots induced for $NO_3^-$ uptake. - Plant Physiol. **109**: 1277-1283, 1995.

Van der Werf, A., Kooijman, A., Welschen, R., Lambers, H.: Respiratory energy costs for the maintenance of biomass, for growth and for ion uptake in roots of *Carex diandra* and *Carex acutiformis*. - Physiol. Plant. **72**: 483-491, 1988.

Warner, R. L., Kleinhofs, A.: Genetics and molecular biology of nitrate metabolism in higher plants. - Physiol. Plant. **85**: 245-252, 1992.