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Compartmentation and fluxes of sugars in roots of *Phaseolus vulgaris* under phosphate deficiency

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Abstract

The influence of phosphate deficiency on the sugar accumulation and sugar partitioning in the root cells of bean (*Phaseolus vulgaris* L.) was studied. Bean plants were cultured 17 - 19 d on a phosphate-sufficient and phosphate-deficient nutrient medium. Phosphate deficit in the growth medium resulted in increased sugar concentration for about 30 % in the apoplastic and cytoplasmic compartments as well as in the vacuoles of root cells. However, the distribution of sugars between apoplast and cytoplasm compartment and vacuole was not affected by decreased phosphate concentration. About 20 % of sugars were found in the apoplast and cytoplasm, about 80 % in the vacuole. Low phosphate concentration enhanced influx of exogenous ^{14}C-sucrose into meristematic and elongation zones of root. The ^{14}C-labelled sugar content in the root tips increased for about 60 % as compared to control plants. Phosphate deficiency increased also ^{14}C-glucose uptake and content in the root tips. However, the amount of $^{14}CO_2$ liberated during respiration of P-deficient roots (after feeding with uniformly labelled ^{14}C-glucose) was lower than $^{14}CO_2$ respired by control plants, thus a large part of accumulated sugars seems to be metabolically inactive.

Additional key words: apoplast + cytoplasm, ^{14}C-glucose, ^{14}C-sucrose, sugar localization, vacuole.

Introduction

One of the most visible symptoms of phosphate deficiency is the increase in the root/shoot mass ratio which is the result of shoot growth reduction and stimulation of root growth (Fredeen *et al.* 1989, Rao and Terry 1989, Khamis *et al.* 1990). The

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increase in root fresh and dry mass is associated with the higher sugar content in the roots and is considered to be an acclimation of plants to the low external concentration of P (Fredeen *et al.* 1989, Cakmak *et al.* 1994a, Rychter and Randall 1994). Accumulation of soluble sugars in the roots of P-deficient plants is the result of increased assimilate transport from the shoot to the root (Cakmak *et al.* 1994b, Ciereszko *et al.* 1996) and also to some extent the increased sucrose hydrolysis in the root (Ciereszko *et al.* 1998), decreased hexose phosphorylation (Rychter and Randall 1994) and decreased respiration rate of whole roots (Rychter and Mikulska 1990).

Little is known about compartmentation and function of accumulated sugars in the roots of P-deficient plants. It was suggested that at low nutrient supply soluble sugars were all located in the vacuoles of *Plantago major* roots and as such not available for respiration (Lambers 1985). Observations of ultrastructure of cells from meristematic region of P-deficient roots indicated increased vacuolization and plasmalemma invaginations as a possible place for sugar accumulation (Wanke *et al.* 1996, 1998).

The purpose of this study was to determine partitioning of sugars in the cell compartments of the roots at low phosphorus supply. The effect of P-deficiency on ^{14}C-sucrose and ^{14}C-glucose uptake by the root segments and participation of labelled glucose in respiration was also estimated.

Materials and methods

Plants: Bean (*Phaseolus vulgaris* L. cv. Gold Saxa) plants were cultured hydroponically in containers with complete nutrient medium (+P, control) or without phosphate (-P) as described previously by Ciereszko *et al.* (1996). Plants were grown in the growth cabinet (*Fi-totron PG660, Sanyo Gallenkamp,* Leicester, UK) at day/night temperature of 20/18 °C, and 16-h photoperiod (photon flux density of 400 μmol m^{-2} s^{-1}. Plants at the age of 17 - 19 d were harvested after 4 - 5 h of light period. Inorganic orthophosphate (Pi) in the roots was assayed by the Fiske-Subbarow method as described by Rychter and Mikulska (1990).

Sugar compartmentation: The compartmentation of soluble sugars was estimated by method of an isotope washout (Farrar 1985). The advantage of such analysis is that high-level of organization within the tissues is not disrupted during the experiments, although the assignation of localization to the distinct kinetic pools is indirect (Pollock and Kingston-Smith 1997). ^{14}C-sugars efflux from intact whole roots of +P and -P bean plants was measured after 63 h pulse-labelling feeding of $^{14}CO_2$ to the first leaves. Isotope labelling for 63 h was long enough to produce uniform specific activity of soluble sugars in the roots. Roots were rapidly removed and placed in 3 cm^3 of 20 mM sucrose solution containing 0.1 mM $CaSO_4$. This allowed the exchange of endogenous ^{14}C-sucrose (in the roots) with exogenous sucrose (in the incubation medium). The roots were transferred serially through 25 changes of sucrose solution at temperature 20 °C, at various times over the next 8 h. After that time the radioactivity of each change of solution and the extract of root tissue with 90 % ethanol, were counted by liquid scintillation.

Two phases of isotope loss from +P as well as from -P roots were observed (data not shown). The faster had a half-time of about 30 - 40 min and was ascribed to apoplastic + cytoplasmic pool of sugars, the slower one was ascribed to washout from the vacuole (Farrar 1985).

For sugar determinations plant material was extracted as described Farrar and Farrar (1985). Sugars in the extracts were measured by the phenol-sulphuric acid method (Dubois *et al.* 1956).

Apoplastic volume was calculated as described by Gifford and Thorne (1985) and Tetlow and Farrar (1993). Sixty root tips (2 cm length) were cut from 17-d-old +P and -P plants, washed with 1 mM $CaCl_2$, weighted and placed in 7 cm^3 of nutrient solution (+P or -P) containing 50 mM MES, pH 6.0, 20 mM PEG 1 000 and ^{3}H-PEG 900 (74 kBq cm^{-3}) in small Petri dishes which were shaken at 20 °C for 2 h. At various intervals three root tips were removed, washed for about 5 s, dried for 5 - 10 s and transferred to vials with *Scintran*, tissue solubilizer (Tetlow and Farrar 1993).

^{14}C-sucrose feeding: Sixty root tips (2 cm length) were cut, washed in 1 mM $CaCl_2$, weighed and placed in 5 cm^3 of nutrient solution (+P or -P) containing 50 mM MES, pH 6.0, 2 mM (or 20 mM) sucrose-carrier and 370 kBq of ^{14}C-sucrose. The root tips were incubated in small Petri dishes at 20 °C during 3 h and at various times 3 root tips were removed, washed for 5 s, dried 5 - 10 s and then transferred to vials with tissue solubilizer. After 3 h of incubation, remained root tips were rinsed with cold 1 mM $CaSO_4$ and placed into medium (as described above but without ^{14}C-sucrose) for next 4 h. During that time ^{14}C-sucrose remained in the root tips was measured at various intervals, after tissue solubilizer treatment. Sucrose was extracted from the root tips as described by Fredeen *et al.* (1989) and after digestion by invertase was estimated enzymatically with glucose oxidase and peroxidase (*Sigma-Aldrich*), as described by Rychter and Randall (1994).

^{14}C-glucose feeding: Thirty root tips (1.5 cm length) were cut, washed in 1 mM $CaCl_2$, weighed and placed in 2 cm^3 of nutrient solution (+P or -P) containing 10 mM MES, pH 6.0, 10 μM glucose-carrier and 370 kBq of ^{14}C-glucose in small bottle (volume 10 cm^3) then were shaken for 20 min at 20 °C (according to Beevers 1991). Respired $^{14}CO_2$ was trapped by *Whatman No. 1* paper (2 × 2 cm) moistened with 10 % KOH.

Radioactivity determination: Radioactivity of a sugar fraction was measured in ethanol extracts, total radioactivity was measured after overnight soaking in tissue solubilizer (*Scintran*). All samples were added to *Aquasol* (*New England Nuclear*, UK) and radioactivity was counted using *LKB Rackbeta 1209* liquid scintillator counter (*LKB Pharmacia*, Uppsala, Sweden).

All data presented are the means of three to four experiments ± standard deviation.

Results and discussion

Root growth and Pi concentration: Bean plants after 17 - 19 d of culture on phosphate-deficient (-P) nutrient medium showed typical symptoms of phosphate deficiency: content of inorganic phosphate (Pi) decreased in the tissues but root growth and sugar concentration in the roots were increased. Pi content in the roots of -P plants decreased to about 5 % of the control [+P: 0.59 ± 0.08; -P: 0.028 ± 0.003 mg g^{-1}(f.m.)]. The fresh mass of -P root was increased for about 60 %, as compared to the control plants (+P: 0.9 ± 0.1; -P: 1.5 ± 0.2 g $root^{-1}$). The length of roots of -P plants increased for 34 % (+P: 20.5 ± 2.0; -P: 27.5 ± 2.5 cm). These changes were accompanied with the increased sugars concentration in -P roots similarly as observed previously (Ciereszko *et al.* 1996, 1998, Wanke *et al.* 1998).

Sugar pools in the roots: The proportion of sucrose in apoplast and cytoplasm (fractions which were first washed out from the roots) to vacuolar pools was similar in -P and +P plants; about 20 % of sugars were found in the apoplast and cytoplasm and about 80 % in the vacuole (Table 1). However, despite that the sugars distribution was similar, the total radioactivity of sugars in -P roots was higher in apoplast + cytoplasmic pool for about 60 % and in vacuolar pool for 55 % (Table 1). Unlabelled sugars concentration in apoplast + cytoplasm and vacuole of the whole roots of -P plants increased for about 32 % and 27 %, respectively (Table 1).

Table 1. Pools of soluble sugars estimated from efflux of ^{14}C-sugar from roots of plants previously labelled to constant specific activity. Bean plants were cultured 19 d on phosphate-sufficient (+P) and phosphate-deficient (-P) nutrient medium. Means (n = 4) ± SD.

Sugar pools	+P		-P	
	apoplast + cytoplasm	vacuole	apoplast + cytoplasm	vacuole
Radioactive sugars distribution [%]	20.2	79.8	20.7	79.3
Sugar fraction radioactivity [kBq g^{-1}(d.m.)]	29.35 ± 5.06	115.96 ± 19.90	47.06 ± 8.22	180.29 ± 31.47
Sugar content [mg g^{-1}(d.m.)]	21.47 ± 0.61	84.83 ± 2.60	28.26 ± 1.32	108.24 ± 4.37

Depending on the plant species and the metabolic condition of the plant, the vacuolar proportion of sucrose may vary; values ranging from 20 to 100 % of the total cellular sucrose content have been reported (Martinoia and Ratajczak 1997). In barley roots cytoplasmic and apoplastic sucrose pool accounts for 40 % of total sucrose (Farrar 1985). In the roots of P-deficient beans the reducing sugars content increased mainly in the cytosol, while the most of sucrose was detected in the vacuoles (Wanke *et al.*1998).

The presence of numerous plasmalemma invaginations and secondary vacuoles was observed in the periblem cells from root tips of -P bean plants (Wanke *et al.*

1996, 1998). These observations together with estimation of soluble sugars released after 10 % DMSO treatment indicate that invaginations of plasma membrane might be also a possible place for sugars accumulation (Wanke *et al.* 1998).

Apoplastic volume: Plasmalemma invaginations observed in the cells of -P root could increase apoplast volume (defined as the intercellular space and cell wall). In this paper the calculations of apoplast volume of bean root were done by saturating the free space with a slightly membrane permeable ^{3}H-PEG-900, as described by Tetlow and Farrar (1993). Incubation of root segments in the medium with ^{3}H-PEG-900 solution resulted in a rapid diffusion of the tracer into the apoplast (within 15 - 20 min in both group of plants) followed by a slow linear rate of diffusion into intracellular compartments (Fig. 1). The mean rates of ^{3}H-PEG-900 uptake were 0.258 and 0.347 nmol g^{-1}(f.m.) s^{-1} for +P and -P plants, respectively. The fast slope of the curves allowed for an approximate estimations of apoplastic volume in the root tips: for +P plants about 0.12 cm^3(^{3}H-PEG) g^{-1}(f.m.) and for -P plants about 0.16 cm^3(^{3}H-PEG) g^{-1}(f.m.), but differences were statistically insignificant. Volumes of the apoplast presented as percentage of tissue water content were 13 % and 17 % for +P and -P plants, respectively.

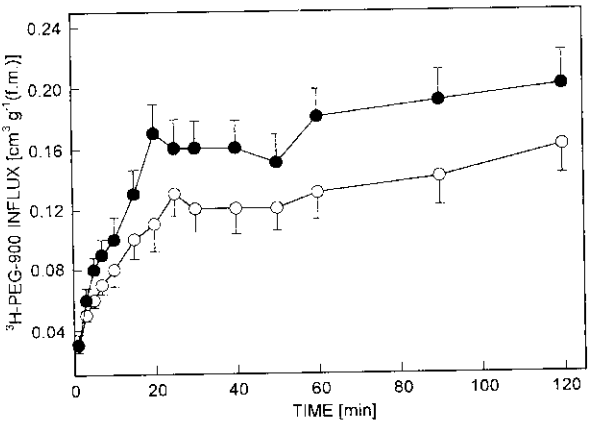

Fig. 1. Influx of ^{3}H-PEG-900 into root tips during 3 h incubation with ^{3}H-PEG-900. Bean plants were cultured 17 d on P-sufficient (+P; *open circles*) and P-deficient (-P; *close circles*) medium.

Phosphate deficiency did not change significantly an influx of ^{3}H-PEG-900 and volume of apoplast in +P and -P root tips. It can be explained by the differences in the cell ultrastructure between -P and +P plants which were observed only in periblem cells of bean root (Wanke *et al.* 1996, 1998) and the calculated apoplast volume reflects the value for all tissues in the root.

Ho and Gifford (1984) found that PEG-900 was not transported into the symplast of wheat grains but accumulated in the apoplast and suggested that the same apoplastic volume might be available to sucrose. The comparisons of ^{3}H-PEG-900 influx and ^{14}C-sucrose influx or ^{3}H-inulin efflux and unlabelled sugar efflux were made by other authors to estimate sugar content in apoplast of soybean cotyledons

and pericarp discs of tomato fruits, respectively (Gifford and Thorne 1985, Damon *et al.* 1988). From the influx kinetics of ^{3}H-PEG-900 an apoplastic influx time of about 15 - 20 min was determined (Fig. 1). Similar time of ^{14}C-sucrose influx could indicate the diffusion of this sugar into apoplastic space.

Exogenous ^{14}C-sucrose uptake by root tips: Phosphate deficiency increased uptake of sucrose and labelled sugar content in root tips for about 60 % after 3 h incubation in the medium with 2 mM sucrose and ^{14}C-sucrose (185 kBq and 2 mM sucrose-carrier) (Table 2). The concentration of unlabelled sucrose in the root tips -P plants was higher for about 70 % than in the control plants [+P: 0.45 ± 0.07, -P: 0.76 ± 0.08 mg g^{-1}(f.m.)].

Table 2. ^{14}C-sugar content and uptake of sucrose into root tips after 3 h incubation with ^{14}C-sucrose (185 kBq + 2 mM sucrose carrier). Bean plants were cultured 17 d on phosphate-sufficient (+P) and phosphate-deficient (-P) nutrient medium. Means (n = 3) ± SD.

Sugar content and uptake	+P	-P
^{14}C-sugar content [kBq g^{-1}(f.m.)]	27.1 ± 1.9	44.2 ± 3.2
^{14}C-sucrose uptake [μg g^{-1}(f.m.) s^{-1}]	0.047 ± 0.003	0.075 ± 0.005

The influx of labelled sucrose had two main phases: fast phase of up to 30 min, followed by slower phase (Fig. 2). No differences between +P and -P plants were observed until 1.5 h of incubation. After this time ^{14}C-sucrose content in the root tips of the phosphate-deficient plants increased for about 60 % as compared to the control (Fig. 2*A*). After 3 h, remained root tips were rinsed and placed into incubation medium without ^{14}C-sucrose. During first 15 - 20 min approximately 30 % of incorporated ^{14}C-sucrose was rapidly washed out (Fig. 2*B*). The remaining sucrose

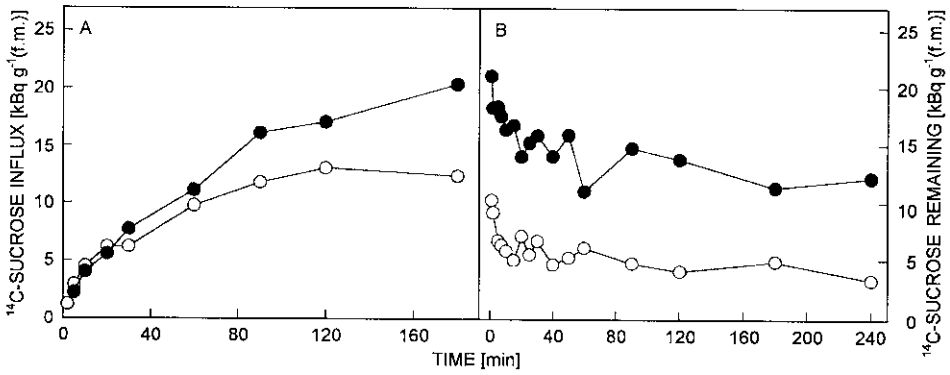

Fig. 2. ^{14}C-sucrose influx into root tips (*A*) during 3 h incubation with ^{14}C-sucrose (370 kBq + 20 mM sucrose carrier) and ^{14}C-sucrose remaining in the root tips after incubation in medium without radioactivity (*B*). Bean plants were cultured 17 d on phosphate-sufficient (*open circles*) and phosphate-deficient (*close circles*) nutrient medium. One typical replicate is shown.

was washed out slowly during next 1.5 h of incubation. After this time ^{14}C-sucrose content in the root segments was constant, however in -P roots it was more than 2 times higher than in the control (Fig. 2*B*). ^{14}C-sucrose that remained in the root segments, was located probably in the vacuoles. Moreover after 2 - 3 h the part of ^{14}C might be incorporated into various compounds of the cell, *e.g.* insoluble products. Enhancement of sucrose uptake by -P root tips is not easy to explain; further studies are necessary to establish the basis of this process. It may be a result of osmotic alteration inside the cell or a changes in membrane permeability to sugars (affected directly or indirectly by phosphate deficiency), *e.g.* the increase in amount/ability of sugar transporters in membrane.

Roots of tobacco plants grown in nutrient-deficient conditions responded to added sucrose by growing more rapidly, despite high concentration of soluble sugars (Paul and Stitt 1993). It was suggested that the plants may sense internal and external carbohydrate differently, implying that there is tight regulation of the metabolism and compartmentation of the soluble carbohydrates in the roots of the nutrient-deficient seedlings (Paul and Stitt 1993).

Utilization of ^{14}C-glucose in root tip metabolism: Short-term feeding of uniformly labelled ^{14}C-glucose to the tissue results in the labelling of mainly cytosolic pool of soluble sugars (Beevers 1991). Uptake of labelled glucose was 31 % higher in root tips of phosphate-deficient than in the control plants (Table 3). After 20 min of feeding of ^{14}C-glucose to root segments, radioactivity of sugar fraction in phosphate-deficient plants increased for about 50 % as compared to the control (Table 3). Nevertheless, the root tips of -P plants released about 30 % less of $^{14}CO_2$ than tips from roots grown on the complete nutrient medium (Table 3).

Table 3. ^{14}C content in root tips after 20 min incubation with ^{14}C-glucose (370 kBq + 10 μM sucrose carrier). Bean plants were cultured 17 d on phosphate-sufficient (+P) and phosphate-deficient (-P) nutrient medium. Means ($n = 4$) ± SD.

Sugar utilization	+P	-P
^{14}C-glucose uptake [nmol g^{-1}(f.m.) s^{-1}]	1.44 ± 0.17	1.89 ± 0.19
^{14}C in sugars fraction [kBq g^{-1}(f.m.)]	137.4 ± 21.0	205.7 ± 16.0
^{14}C in insoluble fraction [kBq g^{-1}(f.m.)]	12.6 ± 2.3	9.1 ± 3.5
Respired $^{14}CO_2$ [kBq g^{-1}(f.m.)] .	26.4 ± 6.5	17.6 ± 1.4

The decrease of $^{14}CO_2$ respired by the roots of phosphate-deficient plants, compared to the control, indicated that a large part of accumulated sugars in the -P roots might be a metabolically inactive pool of sugars then also not used to the root growth. In the roots of -P bean the ratio of unphosphorylated to phosphorylated sugars was about five to seven times higher than in +P roots (Rychter and Randall 1994). Moreover it was shown that glucose accumulated in the root tips during phosphate deficit as well as glucose fed into phosphate-deficient roots did not increase the respiration rate (Wanke *et al.* 1998). Therefore it seems that sugars

accumulated in the roots in response to Pi deficit in growth media may serve other functions, *e.g.* as osmoregulants increasing osmotic pressure of cells and eventually their uptake capacity.

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