ASSIMILATE DISTRIBUTION IN BEAN PLANTS (*PHASEOLUS VULGARIS* L.) DURING PHOSPHATE LIMITATION

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ABSTRACT

The influence of phosphate deficiency on the increased ¹⁴C-assimilate transport from shoot to root of bean plants (*Phaseolus vulgaris* L.) was studied. The roots of plants were cultured in split configurations (a half of the root system was exposed to a short-term or long-term culture in phosphate-deficient nutrient medium, while the other half – in complete nutrient medium) to establish the conditions of translocation enhancement.

It was found that both short term Pi stress applied to a part of root and longer localized phosphate deficiency is not sufficient to increase assimilate transport from the shoot to the root. Low concentration of Pi in tissues of the whole plant as a signal for changes in assimilate distribution and sugar accumulation in the roots is discussed.

KEY WORDS: bean, ¹⁴C-assimilate, Pi, split root system, sugars accumulation.

INTRODUCTION

Culture of bean plants for 2-3 weeks on nutrient medium without phosphate has no effect on net photosynthesis rate, but increases ¹⁴C-assimilate translocation from the shoot to the root (Ciereszko et al. 1996). Analysis of phloem exudate from phosphate-deficient leaves of bean indicated also the stimulation of sucrose export (Cakmak et al. 1994b). As a result of enhancement of assimilate transport from bean leaves the increase of carbohydrate content in the roots of -P plants was observed (Cakmak et al. 1994a; Rychter and Randall 1994; Ciereszko et al. 1998). The greater assimilate transport and sugar accumulation in the roots appears to be an early plant response to phosphate deficiency and may be involved in plant acclimation to low nutrient conditions. It was suggested that phosphate-deficient plants may allocate more photosynthates from the leaves to the roots to improve their capacity for absorbing phosphate ions (Fredeen et al. 1989; Jeschke et al. 1997). However prolonged drastic reduction of Pi concentration in plant tissues leads often to a lowering of the assimilate production rate and transport of assimilate (Hart and Greer 1988).

The aim of a present study was to establish whether the signal for increased assimilate translocation results from temporary Pi deficiency in nutrient medium or significant decrease of Pi concentration in root tissues. We studied the distribution of incorporated ¹⁴C between shoot and roots on single plants applying split root system to differentiate Pi concentration in the roots of the same plant.

MATERIAL AND METHODS

Plant material

Bean (*Phaseolus vulgaris* L. cv. Złota Saxa) seeds were germinated for 2 days at room temperature and then the primary root tips were partially removed as described by Schumacher and Smucker (1985). This resulted in a split root system composed of laterally developed roots from main axis of plant. After 7 days the plants were transferred into a double vessel (split root chambers); each compartment of the vessel contained 0.5 l of nutrient solution. Two plants were fixed in each double vessel with separated halves of the split root system in each compartment. The separate compartments of the chamber were filled with complete Knop nutrient medium (+P) or with Pi-deficient medium (-P); as a control some of the chambers were filled only with +P or with -P nutrient solution, so the whole root system was growing in the same medium. The composition of nutrient solution and the growth conditions were the same as described by Ciereszko et al. (1996).

Pi estimation

Inorganic phosphate (Pi) was determined directly after homogenization and extraction of roots with cold 10% TCA using Fiske-Subbarow method as described by Rychter and Mikulska (1990).

Assimilate distribution

The radioactivity in the shoots, whole roots or in both parts of the split root system was measured after 20 min of ¹⁴CO₂

incorporation into leaves and 4 h of translocation in the light as described by Ciereszko et al. (1996). ^{14}C labelling of ethanol soluble products from shoots and roots was estimated using bean plants:

a) cultured in split-root conditions for 8 days on +P or -P nutrient medium, 4 h before experiment a half of the root system from +P was transferred into -P nutrient medium (short-term phosphate deficiency, 8 hours on -P nutrient medium) or a half of root system from -P was transferred into +P nutrient medium (Fig. 1);

b) cultured for 8 days in split-root system conditions (+P/-P) (long-term of phosphate deficiency);

c) cultured for 8 days in whole root system on +P or on -P nutrient solution (controls) (Fig. 1).

Fig. 1. Schematic display of different versions of experiment, in which assimilate distribution was estimated; details – in text of Material and Methods.

Sugar determination

For sugar extraction fresh samples of leaves and roots (200 mg) were frozen in liquid N then ground in 80% ethanol and incubated at 40°C for 18 h (Fredeen et al. 1989). Reducing sugars content was determined using Somogyi-Nelson method (Nelson 1944).

All determinations were performed in three replicates in three to five independent series of experiments and standard deviation (SD) was calculated.

RESULTS

Exposition of a half of the bean (*Phaseolus vulgaris* L.) root system to phosphate-deficient nutrient medium did not affect shoot and root growth parameters (Fig. 2A). Plant cul-

Fig. 2. Fresh weight of shoot and parts of root of bean plants cultured for 4, 6 and 8 days in a split root system (A) or in a whole root system (B); means $\pm$ SD.

ture on phosphate-deficient medium of a whole root system (as control to split root system conditions) showed typical symptoms of phosphate deficiency: after 8 days the shoot fresh mass was about 30% lower and root fresh mass was about 50% higher as compared to phosphate-sufficient plant (Fig. 2B).

The removal of phosphate from the nutrient medium decreased inorganic phosphate (Pi) in the exposed half of bean root while in the leaves and the other half of root Pi content was almost constant (Fig. 3A). Pi concentration in the part of root cultured on the phosphate-deficient medium decreased after 4 days by about 60%, after 6 days – by 86% and after 8 days was only 8% of Pi content in the half of root system that was grown in the phosphate-sufficient medium (Fig. 3A). Comparatively, after 8 days of plant culture on phosphate deficient medium of a whole root system (as control to split root system) the Pi content decreased both in leaves and roots of -P plants: in the leaves was lower by about 90% and in the whole roots only 4% of that grown on phosphate-sufficient medium (Fig. 3B).

Short time exposure of halves of root system to -P or +P nutrient medium did not affect significantly ^{14}C -assimilate distribution (ethanol soluble fraction) between shoot and root. Transfer for 8 hours to -P medium of a half of root system from plants grown for 8 days on +P nutrient medium resulted only in a small change (statistically not significant) in distribution of ^{14}C -assimilates between shoot and root. In such conditions about 84% of ^{14}C -assimilates was located in shoot and about 16% in both parts of root (+P – 8.7%, -P – 7%) whereas in control experiment (both halves of root placed in +P medium) 90% was in shoot and 10% in roots (Fig. 4A, B).

Fig. 3. Inorganic phosphate (Pi) concentration in leaves and roots of bean plants cultured for 4, 6 and 8 days in a split root system (A) or in a whole root system (B); means $\pm$ SD.

Transfer for 8 hours to +P medium of a half of the root system from plants grown for 8 days on -P nutrient medium had no effect on distribution of radioactivity between shoots and both parts of root (Fig. 4C, D). About 76% of ¹⁴C-assimilates

Fig. 4. Distribution of radioactive carbon (as% of radioactivity in water soluble fraction) between shoot and parts of root of bean plants cultured: A) 8 days on phosphate-sufficient medium and for 8h a half of root system was transferred into phosphate-deficient nutrient medium; B) all time on phosphate-sufficient medium; C) 8 days on phosphate-deficient medium and for 8h a half of root system was transferred into phosphate-sufficient nutrient medium; D) all time on phosphate-deficient medium.

The values of ¹⁴C labelling (kBq g⁻¹FW) were: A) shoot 50.6 $\pm$ 4.4, +P root 5.2 $\pm$ 1.2, -P root 4.2 $\pm$ 0.6; B) shoot 53.8 $\pm$ 4.2, +P root 2.7 $\pm$ 0.6, +P root 3.3 $\pm$ 0.7; C) shoot 54.6 $\pm$ 4.4, +P root 8.9 $\pm$ 1.2, -P root 8.1 $\pm$ 1.6; D) shoot 55.7 $\pm$ 4.3, -P root 8.9 $\pm$ 1.1, -P root 9.6 $\pm$ 1.5.

Fig. 5. Distribution of radioactive carbon (as% of radioactivity in water soluble fraction) between shoot and root of bean plants cultured for 8 days in a split root system (+P/-P) and for 8 days in a whole root system on phosphate-sufficient (+P) or on phosphate-deficient medium (-P). The values of ¹⁴C labelling (kBq g⁻¹FW) were: +P/-P plants: shoot 94.8 $\pm$ 14, +P root 6.6 $\pm$ 1.7, -P root 8.8 $\pm$ 2.5; +P plants: shoot 101.1 $\pm$ 18, root 16.5 $\pm$ 5; -P plants: shoot 114.1 $\pm$ 29, root 44.3 $\pm$ 17.

was located in shoot and about 24% in both parts of root (+P – 12.4%, -P – 11.3%), whereas in control experiment (both halves of root placed in -P medium) 75% was in shoot and 25% in roots (Fig. 4C, D).

Partitioning of ¹⁴C-assimilates between halves of root of a bean plant cultured for a long time (8 days) in split root conditions (one part of root in phosphate-sufficient, other part – in phosphate-deficient nutrient medium) was similar (Fig. 5). In shoots of such plants detected were 86% of ¹⁴C-assimilates, in the +P half of root – 6% and in the -P half of root – 8%, such pattern of distribution was similar to that in plants grown on +P nutrient medium (whole root system) (Fig. 5). After 8 days of bean plant culture on phosphate-deficient nutrient medium (whole roots exposed) an about 2-fold increase of ¹⁴C-labelling in ethanol soluble assimilate fraction from the roots (after 4h translocation in the light) was observed, compared with +P plants (Fig. 5). In -P plants in the shoot were 72% of ¹⁴C labelled assimilates and 28% were in the root, whereas in +P plants in the shoot was located about 86% and in the root – 14% (Fig. 5).

As there was no significant difference in assimilate transport from the leaves to both the parts of root the reducing sugars content in the parts of split root system grown in phosphate-sufficient or in phosphate-deficient nutrient medium was also similar (Fig. 6A). On the other hand, the increase of assimilate transport from shoot to root was mainly responsible for higher content of soluble sugars in whole roots of -P plants – after 8 days of culture reducing sugar content in the leaves of -P plants was higher for 56% and in the roots – for 90% as compared with control (Fig. 6B).

DISCUSSION

Split root experiments, in which the plants were cultured with part of their root system in high phosphate and the other in low phosphate medium have shown that growth, sugar concentration, as well as the radioactivity and distribution of ¹⁴C-assimilates were similar for +P and -P halves of divided root (Figs 4-6). These results indicate that both temporary (hours)

Fig. 6. Reducing sugars content in the leaves and parts of roots of bean plants cultured for 4, 6 and 8 days in a split root system (A) or in a whole root system (B); means $\pm$ SD.

and long-term (days) removal of Pi from nutrient solution surrounding only a part of root (localized P deficiency) is not an adequate signal for the increased translocation and changes in assimilate distribution. Drastic decrease of Pi concentration in plant tissues, probably not only in the root but also in the shoot, is necessary to induce the higher sucrose transport and sugar accumulation in the root.

The split root method was used in several studies to investigate the impact of an uneven distribution of nutrient, mainly nitrogen, but also phosphorus, on ions uptake by root (Schortemeyer et al. 1993; Agrell et al. 1994; Chaillou et al. 1994; Cogliatti and Clarkson 1983). In potato plants, when roots were divided between solutions with or without phosphate, the increased absorption rate of ³²P was determined not by the P-status of the one part of root, but by the general demand for phosphate in the plant (Cogliatti and Clarkson 1983). Phosphorus is a nutrient that can be retranslocated within the plant, e.g. from old leaf to the younger leaves (Marschner et al. 1997), however in our studies the transport of Pi between two divided parts of the root system seems negligible. Split root experiments in barley have shown that carbohydrate transport is sink-regulated and responds to total capacity of carbon utilization (Farrar and Minchin 1991).

Pi concentration in roots of bean plants grown for 8 days in phosphate-deficient medium (whole root exposed) dropped to 4% of the control and in these conditions the increased assimilate translocation to roots was observed (Fig. 3, 5). The ¹⁴C-labelling in roots was the highest in ethanol soluble-sugar fraction, the labelling of amino acids and organic acids fractions were markedly lower than in control roots (Ciereszko et al. 1996). The highest sugar concentration was detected in the

meristematic and elongation zones of bean root and those sugars were probably accumulated in plasmalemma invaginations and secondary vacuoles (Wanke et al. 1998). These results, together with the observed lower level of phosphorylated sugars (Rychter and Randall 1994) and the failure of glucose to increase respiration rate in phosphate-deficient roots, indicate that accumulated sugars are not used in respiratory metabolism (Wanke et al. 1998; Ciereszko et al. 1999).

It has been suggested that the accumulated sugars may serve as osmoregulators, increasing osmotic pressure of cells and eventually their uptake capacity (Rychter and Randall 1994; Ciereszko et al. 1996). The mechanism and significance of Pi deficiency induced increased sugar transport and as a consequence induced sugar concentration in the roots is not entirely understood. The action of plant hormones may be involved in regulation of assimilate partitioning, similarly as it was observed under other stresses (Starck and Cieřla 1989).

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DYSTRYBUCJA ASYMLATÓW W ROŚLINACH FASOLI (*PHASEOLUS VULGARIS* L.)
PODCZAS DEFICYTU FOSFORU

STRESZCZENIE

Badano wpływ deficytu fosforu na wzrost transportu ^{14}C -asymilatów z pędu do korzenia fasoli (*Phaseolus vulgaris* L.). Miejsce percepcji sygnału zwiększającego przepływ asymilatów ustalano rozdzielając system korzeniowy roślin (połowę systemu korzeniowego umieszczano na krótki lub dłuższy czas w pożywce pozbawionej fosforu a połowę – w pożywce pełnej).

Stwierdzono, że zarówno krótkotrwały jak i długotrwały stres fosforanowy tylko w części korzenia nie jest wystarczający do wzmożenia transportu asymilatów z pędu do korzenia. Niskie stężenie P_i w tkankach całej rośliny jest niezbędne do zapoczątkowania zmian w dystrybucji asymilatów i gromadzenia cukrów w korzeniu.

SŁOWA KLUCZOWE: fasola, ^{14}C -asymilaty, P_i , rozdzielony system korzeniowy, gromadzenie cukrów.