Plant Science Letters, 35 (1984) 81—85
Elsevier Scientific Publishers Ireland Ltd.

QUANTIFICATION OF THE XYLEM-TO-PHLOEM TRANSFER OF AMINO ACIDS BY USE OF INULIN [^{14}C]CARBOXYLIC ACID AS XYLEM TRANSPORT MARKER

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(Received August 25th, 1983)
(Revision received January 7th, 1984)
(Accepted January 7th, 1984)

Inulin [^{14}C]carboxylic acid and ^{14}C-labelled amino acid (α-aminoisobutyric acid (aib) and valine) solutions were introduced into the transpiration stream through the cut stem bases of young (4—12 leaves) tomato plants. Inulin carboxylic acid (inu) was translocated exclusively by the xylem, whereas the amino acid distribution resulted from both xylem and phloem import. Comparison of the distribution of inu and aib permitted a quantitative assessment of the xylem-to-phloem transfer in the stem.

Of aib, 20.6% traversed from xylem to phloem in a plant with 12 leaves. The phloem import was not evenly distributed over the leaves and varied from 0% (first five leaves) to 95% (top leaf) of the aib import per leaf. Doubling the flow rates in the xylem reduced the aib supply to 25% in the top leaf and 55% in the next leaf, which reflects a reduced xylem-to-phloem transfer.

Key words: Lycopersicon esculentum; amino acid uptake; xylem-to-phloem transfer

Introduction

Ringing experiments [1] and the high amino acid and amide content in the bleeding sap of various plants [2] indicated the xylem as the main upward translocation path for nitrogenous substances. Later studies have shown that amino acids traverse from the xylem to the phloem along the path [3—8]. This radial passage is an important step in the nitrogen economy of the plant. Xylem-to-phloem transfer in *Lupinus* [7] provides the vegetative apices, fruits and the nodulated roots with 60, 70 and 23%, respectively, of the nitrogen received through the phloem.

A primary question on lateral transfer is the route of transport. In *Lupinus albus* [5] several amino compounds (valine, asparagine, threonine, serine, citrulline and glutamine) pass from the xylem to the phloem in an unchanged form. Other amino acids (glycine, methionine, aspartic acid, glutamic acid and aib) are strongle transformed during the passage. The metabolic transformations probably take place before entrance into the phloem and thus suggest symplastic transport being involved in the lateral transfer. Ray cells would be the obvious path for this transport. In contrast with this, it was demonstrated that an apoplastic xylem-to-phloem route could account for the lateral amino acid transfer [9].

Another open question is how amino acids enter the symplastic network. Some evidence for a coupled proton-amino acid escape from the xylem vessels has been reported [10] suggesting proton/amino acid co-uptake into the symplastic compartments around the xylem vessels.

Only a rapid method for the quantification of the xylem-to-phloem transfer may permit a valuable experimental approach to the preceding questions. The aim of this study was to develop a simple method to quantify the xylem-to-phloem transfer. Essentially, the method includes comparison of inu (a xylem transport marker) and amino acid distribution between the leaves.

Abbreviations: aib, α-aminoisobutyric acid; inu, inulin carboxylic acid.

0304-4211/84/$03.00
Printed and Published in Ireland

Material and methods

Tomato plants (*Lycopersicon esculentum* cv. Moneymaker) were cultivated in containers with aerated Hoagland solutions. The roots were removed under water and the detached plants (4—12 leaves, dependent on the experiment) were placed in nutrient solutions with ^{14}C-labelled substances. After uptake, driven by the transpiration for 2 h, the plants were immediately cut into pieces which were weighed and subsequently digested in a mixture of 1 ml 70% perchloric acid, 30% hydrogen peroxide (1:1) for 18 h. The ^{14}C-activity of the digests was radioassayed in 9 ml Lumagel (Lumac, Netherlands) by liquid scintillation spectrometry. Calibration curves of the rates of the interconversion of the ^{14}C-compounds into $^{14}CO_2$ were made, from which the initial ^{14}C-content of the digests were calculated.

The radioactive compounds (Radiochemical Centre, Amersham, U.K.) were all uniformly ^{14}C-labelled and their specific activities were 1.85 MBq $mmol^{-1}$, the one exception in specific activity being inulin [^{14}C]carboxylic acid (2.41 MBq $mmol^{-1}$).

Results

In preliminary experiments, the distribution patterns of amino acids appeared to be similar in intact plants and plants without roots (Table I). The large mature leaves contained the highest absolute amount of radioactivity (Table II), whereas the apical leaves were labelled to a high specific activity (dpm/mg lamina tissue). In a 'long-term' experiment (6 h), the ^{14}C-content of the leaves increased proportionally during the first 3 h after the start of the experiment. It can be concluded that the redistribution was of no importance during the normal experimental period of 2 h. Aib (no conversion during translocation through the roots to the stem vessels, Table III) and valine (7% conversion, Table III) proved to be suitable amino acids for comparative distribution experiments between detached and intact plants (Tables I and IV). Inu and aib were used for quantifying xylem-to-phloem

Table I. Percentual distribution of 1 mmol l^{-1} ^{14}C-labelled L-valine between intact and detached tomato plants (6 leaves). The corrected values for distribution in intact plants are computed from the radioactivity that had passed the roots.

	Intact plants		Detached plants
	Percentage	Corrected percentage	Percentage
Roots	33.1	—	—
Stem	14.6	21.8	21.9
Leaves	52.3	78.2	78.1

Table II. Absolute (dpm) and specific (dpm/lamina fresh wt.) distribution of 1 mmol l^{-1} [^{14}C]valine between the laminae of a detached tomato plant (6 leaves).

	dpm	dpm/lamina fresh wt.
Cotyledon 1	196	3.8
Cotyledon 2	147	2.6
Lamina 1	553	4.5
Lamina 2	969	5.3
Lamina 3	8207	27.2
Lamina 4	21424	88.7
Lamina 5	20101	195.0
Lamina 6	8810	442.7
Top	2602	616.6

Table III. Composition of xylem sap bleeding from cut stumps of tomato plants (6 leaves) to the root of which 1 mmol l^{-1} [^{14}C]valine or [^{14}C]aib were applied. The percentages of amino acids, sugars and organic acids were determined by separation in Dowex 50W-X8 mesh (H^+) columns. The amino acids were were analyzed by paper chromatography (Whatman No. 1; butanol/acetic acid/water = 12:3:5) and subsequent scanning for radioactivity.

Amino acid applied to the roots	Percentual composition of the bleeding sap			
	Organic acids + sugars	aib	Valine	Alanine
aib	0.3	99.7		
Valine	3.0		93.0	4.0

Table IV. Percentual distribution of 1 mmol l^{-1} [^{14}C]valine and [^{14}C]aib supplied to intact tomato plants (6 leaves).

	Valine	aib
Roots	33.1	27.0
Stem	14.6	20.8
Leaves	52.3	52.2

transfer (Tables V and VI; Fig. 1) not showing any conversion in stem and leaf tissues.

For experimental use, detached plants were preferred to intact ones, since the absence of roots facilitated the understanding of the distribution patterns for a number of reasons. The presence of roots impedes a straight-forward analysis of distribution patterns, as the withdrawal of amino acids by the roots is substantial (Table IV). Furthermore, sieve tubes are sealed with callose in detached shoots and the xylem is the only route for initial entry into the plant. Uptake by root phloem cells and phloem translocation cannot obscure the xylem-to-phloem transfer in more apical parts. In addition, root removal permits ready access of inu to the xylem vessels, which otherwise would be excluded because of its impermeable nature.

Table V. Percentual distribution of 10 μmol l^{-1} inulin [^{14}C]carboxylic acid and 10 mmol l^{-1} [^{14}C]-aib between the plant parts of detached tomato plants (12 leaves). The results of three different plants are presented. The numbers in parenthesis represent the volume (ml) of the substance supplied.

	Supplied (ml)		
	inu (5)	inu (10)	aib (10)
	Absorbed (ml)		
	4.29	7.34	8.48
%Radioactivity			
in stem	1.3	1.2	15.5
in petioles	0.9	0.8	13.5
in laminae	97.8	98.0	71.0

Inulin [^{14}C]carboxylic acid promised to be a reliable xylem transport marker on account of free space measurements of the xylem pathway in tomato internodes [9]. The present experiments confirmed this expectation (Table V). The amounts of inu in the stem after uptake by detached plants were less than 1% of the total amount of inu in

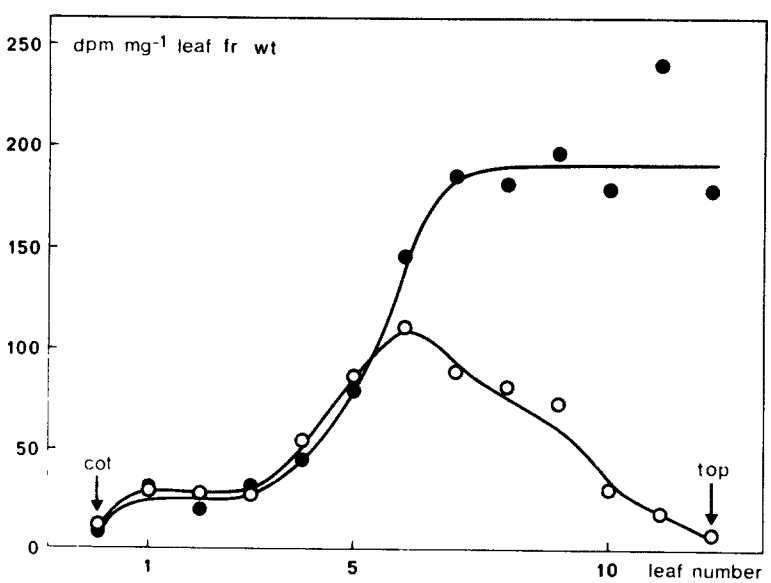

Fig. 1. Distribution of 10 μmol l^{-1} inulin [^{14}C]carboxylic acid (○) and 10 mmol l^{-1} [^{14}C]aib (●) between the laminae in two detached tomato plants (12 leaves).

the plant (Table V). After transpirational washing with water (1 h) in control experiments, less than 0.1% of inu remained in the stem. This indicates that the inu translocation was strictly confined to the xylem pathway. Thus, the specific activity of inu in the leaves provides a measure of the rate of transpiration and xylem import of each specific leaf. Of aib 15—25% and of valine 20—30% was retained in the stem tissues (Tables IV and V). Such high retention rates in stems and petioles demonstrate that, in contrast to inu, amino acids are withdrawn from the xylem stream by surrounding tissues and can cross from xylem to phloem.

The specific activities of the individual leaves from plants 2 and 3 (Table V) were computed by dividing the radioactivity per leaf by its fresh weight and were plotted against the leaf number (Fig. 1). Inu was concentrated in the large mature leaves to which materials are transported exclusively through the xylem (Fig. 1). The latter notion is based on the assumption that inu is the xylem transport marker (Table V) and on the observation that inu and aib were imported to the same rate in the first five leaves (Fig. 1). It was further concluded that in leaves younger than leaf 5 aib was imported via an additional route, probably the phloem (Fig. 1). The aib distribution indicates that the phloem contributed increasingly to aib import with decreasing age of the leaves (Fig. 1). The phloem import varied from 0% (first five leaves) via 20% (sixth leaf) to 95% (top leaf) of the aib import per leaf. For this study it is important to realize that the excess import of aib compared to inu in the younger leaves (Fig. 1) reflects the xylem-to-phloem transfer, since phloem import in the leaves of detached plants is assumed to be dependent on previous xylem-to-phloem transfer. From the dpm in the leaves and the percentual share of xylem and phloem import, computed from Fig. 1, it was calculated that 20.6% of the aib, imported by the leaves, had traversed from xylem to phloem.

Table VI. Percentual distribution of 10 mmol l^{-1} [^{14}C]aib between the parts of detached tomato plants (5 leaves) at two different transpiration velocities. The lamina percentages are expressed as the percentage of the total amount of dpm in the leaves.

	Transpiration rate	
	1030 $mm^3 h^{-1}$	494 $mm^3 h^{-1}$
Roots	8.2	8.2
Stem	12.5	25.3
Leaves	79.3	66.5
Cotyledons	0.3	1.0
Lamina 1	5.4	3.2
Lamina 2	12.1	5.7
Lamina 3	31.5	20.5
Lamina 4	35.3	35.8
Lamina 5	12.8	23.2
Top	2.8	10.6

High transpiration rates evoking high xylem flow rates will, according to the equations for amino acid escape from the xylem vessels [11], decrease the lateral escape and, consequently, xylem-to-phloem transfer. If the postulate holds true that the amino acid distribution between the leaves reflects the xylem-to-phloem transfer, the specific activities of young leaves would readily react to the rate of transpiration. As anticipated, in a plant with a high flow rate, twice as high as in an other plant (494 $mm^3 h^{-1}$; Table VI), the retention in the stem was reduced by 50%. This reduction mirrors diminished uptake by the parenchymatous xylem cells (unpublished results). Lower uptake by ray cells may be related to reduced lateral transfer through the rays and, consequently, to reduced arrival of aib in the sieve tubes. This probably explains the reduction of ^{14}C-supply to 25% in the top leaf and 55% in the next leaf at the higher xylem flow rate (Table VI).

Discussion

This study shows that the specific labelling of the leaves provides an acceptable quanti-

fied estimate of the xylem-to-phloem transfer and an experimental access to investigate its physiology. The results underline the validity of inulin carboxylic acid as a xylem transport marker. An additional support for the reliability of inu is that the large middle leaves (leaves 5,6,7; Fig. 1) contained specific inu activities four to five times higher than the cotyledons. Earlier experiments [12] showed that these leaves transpired four times as fast as the cotyledons.

An absolute prerequisite for the validity of this method is the absence of redistribution during the period of the experiment. A period of 2 h in these experiments met this crucial requirement. In comparable experiments with bushbean [3] organic N was not redistributed within 6—10 h after the N-supply. In detached pea shoots, however, considerable amounts of asparagine were redistributed after 2 h [13].

Diffusional flow through the secondary wall interstices may theoretically account for the xylem-to-phloem transfer [9]. In that case, solutes diffusing out of the xylem fluid would be directly absorbed from the apoplast by the companion cell/sieve tube complexes. More probably [5], the living vessel-associated cells function as an intermediate between xylem and phloem. On account of polar localization of phosphatase activities, symplastic radial transfer of solutes through the ray cells in trees was postulated [14]. The physiology of the radial transfer and the involvement of the ray cells are under current investigation with the aid of the method presented here.

Acknowledgements

The critical reading of the paper by Dr. J. Patrick is highly appreciated.

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