Plant Physiol. (1986) 82, 1019–1023
0032-0889/86/82/1019/05/$01.00/0

Amino Acid Composition Along the Transport Pathway during Grain Filling in Wheat[1]

Received for publication March 31, 1986 and in revised form August 14, 1986

DONALD B. FISHER*[2] AND PETER K. MACNICOL
Department of Botany, Washington State University, Pullman, Washington 99164 (D.B.F.), *and Division of Plant Industry, Commonwealth Scientific and Industrial Research Organization, G.P.O. Box 1600, Canberra, A.C.T. 2601 Australia* (P.K.M.)

ABSTRACT

The amino acid composition of endosperm cavity sap and of sieve tube saps from the flag leaf, peduncle, rachis, grain pedicel, and grain were determined for wheat plants just past the mid-half of grain filling. On a mole percent basis, glutamine accounted for almost half of the amino acids in sieve tube sap from the peduncle and ear. Other protein amino acids, plug γ-aminobutyrate, were present in varying, but mostly low (a few mole percent) proportions. The amino acid composition of phloem exudate resembled that of the mature wheat grain. The proportions of amino acids in the endosperm cavity were generally similar to those of the sieve tube sap supplying the grain. Cysteine, however, while virtually absent from sieve tube sap, comprised 1 to 2 mole percent of amino acids in the endosperm cavity, suggesting it is transported in a different form. Also, alanine and, to a lesser extent, glutamate were relatively more prominent in endosperm cavity sap than in the sieve tube sap. Thus, while most amino acids were more concentrated in the sieve tube sap than in the endosperm cavity sap, alanine and glutamate appeared to be moving from the sieve tube to the endosperm cavity in the absence of, or perhaps even against, their concentration gradients.

Assimilate movement from the sieve tubes into sink tissues is often presumed to occur by simple diffusion along concentration gradients. However, there are presently no direct measurements of solute concentration and composition along the phloem transport and associated pathways leading to sink tissues. Such measurements are essential to understanding the role of gradients in driving transport and the possible role of metabolism during transport, to identifying possible control points and in formulating mass balance sheets to describe the process. Owing to the number of compounds involved, these questions are particularly difficult to address where nitrogen transport is concerned. Difficulties in obtaining samples along the pathway, whether from the sieve tubes or from other locations, have been an even more severe limitation. Comparative data on sieve tube exudate and sink composition have provided useful insights into relationships between the two (27, 28). By judiciously combining these with other measurements on lupin, Pate (18) and his coworkers have developed the most thorough analysis available of the nitrogen and carbon economies of a developing sink.

Unfortunately, phloem exudate usually cannot be collected from most plants, especially from crop species. Among the cereals, rice is a significant exception. Japanese investigators have succeeded in collecting (10) and analyzing (6, 7) the contents of sieve tube exudate from the severed stylets of phloem-feeding leafhoppers and planthoppers. Phloem exudate has also been obtained from wheat by Simpson and Dalling (23) and from barley by Tully and Hanson (26); both studies employed the EDTA exudation technique in collecting phloem sap from excised leaves.

The apoplastic space between maternal tissues and the developing embryo has provided another point at which samples may be obtained along the pathway to a sink. This has been accomplished for legumes (reviewed by Thorne [25]) and for corn (21), although concentrations have not been obtained, and for cereal grains (8, 9, 22), where the collection of endosperm cavity sap allows concentration measurements. To date, however, it has not been possible to obtain samples from both points (sieve tube and parent-embryo interface) in the same plant for the purpose of evaluating transport-associated gradients.

In combination with endosperm cavity sap collection, the collection of sieve tube exudate from broken grain pedicels (4) and from severed aphid stylets (3) established on wheat plants has recently provided a flexible means of sampling the transport pathway during grain filling in wheat (4, 5). In those studies, the compositions of the transport fluids were assessed in terms of sucrose, glucose, potassium, and total amino acids. The object of the present study was to provide a more detailed analysis of transport gradients along this pathway in terms of individual amino acids. This information is relevant not only in terms of nitrogen transport, including comparisons between the compositions of the assimilate stream and the grain, but also yields useful comparisons of the relative concentrations of anionic, cationic, and neutral compounds along the pathway, thus providing insight into the possible role of electrical gradients as well as concentration differences in driving transport.

MATERIALS AND METHODS

Plants, Aphids, and Sample Collection. Except for some minor differences, the growth of plants and aphids and the collection of sieve tube and endosperm cavity sap samples were as described in the accompanying paper (4). Most stylet exudate samples, however, were collected from stylets exuding in air. Due to the slow flow rates (about 50–100 ml h^{-1} $stylet^{-1}$), the exudate quickly dried to a tacky glass. After about 5 h of illumination, the exudate was washed off and discarded. Fresh exudate was allowed to accumulate on the epidermis for several hours during the latter part of the illumination period, following which it was collected in a small volume of water and dried on a silanized microscope slide. Grain pedicel phloem exudate, collected under

[1] Supported by National Science Foundation grant PCM 83-04693.

[2] Visiting Scientist at the Commonwealth Scientific and Industrial Research Oorganization Division of Plant Industry under the United States/Australia Cooperative Science Program.

mineral oil, was also dried on silanized microscope slides. The dried exudate was washed with toluene to remove adhering mineral oil.

Endosperm cavity sap was collected from several grains and the pooled sample was ejected under mineral oil where the droplet diameter was measured at ×50 magnification for calculation of its volume. Ten μl of water were added, mixed well, and the sample was drawn into a disposable micropipet and frozen until analysis.

Comparison of Stored and Frozen Samples. For stylet exudate, a large number of exuding stylets (about 30) were established on a peduncle and the ear was removed. Exudation was allowed to continue for 20 min, after which the exudate was collected, dried on a silanized microscope slide, and stored at room temperature (22°C) for 3 d. After collecting the first exudate sample, the terminal 5 cm of the peduncle, including the exuding stylets, was submerged in mineral oil. Exudate accumulating during the next 20 min was collected in a fine-tipped pipet and immediately frozen.

For comparison of endosperm cavity sap samples, the cavity contents of several grains were collected during a 10 min interval, mixed thoroughly under mineral oil, and divided into three roughly equal droplets whose diameters were measured. One droplet was dried on a silanized microscope slide and stored at 22°C for 3 d. The second and third droplets remained under mineral oil for an additional 3 h, one at 25°C and the other at 0°C. After the incubation period, the droplets were diluted to an appropriate volume for analysis (see below), drawn into disposable micropipets, and frozen.

Amino Acid Analysis. Samples were first diluted to a total amino acid concentration (10–100 mM) that was suitable for analysis. For endosperm cavity sap samples, the total amino acid content could be estimated adequately from the sample volume (4). Dried stylet exudate samples were dissolved in a small volume of water and ejected under mineral oil. Their osmolality was determined cryoscopically with a nanoliter osmometer and their volume by calculation from the droplet diameter. Amino acids were assumed to account for 25% of the osmolality (2), and dilutions were made accordingly. For analysis of amino acids, the sensitive dual-label dansylation method was used, in which the sample is "spiked" with ^{14}C-labeled amino acids, reduced and carboxymethylated to protect cysteine, then reacted with [^{3}H]dansyl chloride. Individual dansyl amino acids were then isolated by two-dimensional TLC and determined via their $^{3}H/^{14}C$ ratios. The published procedure (15) was modified in several ways. TCA extraction was dispensed with, the sample solution being reduced/carboxymethylated directly. Reaction volumes were decreased to 20 μl, and $LiHCO_3$ buffer (24) was substituted for $NaHCO_3$, which allowed direct spotting of the dansylation mixture without prior cleanup on Porapak resin.

RESULTS

Sample Stability. The amino acid composition of samples given different treatments designed to evaluate sample stability are presented in Table I. The stylet exudate and endosperm cavity sap were obtained at 28 and 21 d post-anthesis, respectively. (Under our conditions, grain filling was complete in about 40 d). There was virtually no difference in amino acid composition resulting from holding dried samples at room temperature for 3 d. (Tryptophan was not detected in the dried cavity sap sample, but almost none was present initially.) Even the incubation of liquid endosperm cavity sap for 3 h at 25°C did not noticeably affect its amino acid composition.

Amino Acid Compositions Along the Transport Pathway. In two experiments, data were obtained for three points along the sieve tube pathway (flag leaf, peduncle, and grain pedicel) and for the endosperm cavity sap of grains from the same plant (Table II). While the flag leaf samples showed a substantial number of differences between experiments, sieve tube exudate obtained from near or within the ear (peduncle, grain pedicels) were similar in composition. Glutamine accounted for almost half of the amino acid content in exudate samples in or near the ear (grain pedicels and peduncle; also, see Tables I and III). Serine was next in importance, with most other protein amino acids accounting for about 1 to 5% each of the total. The sulfur amino acids, cysteine and methionine, were virtually absent, and the aromatic amino acids were also generally quite low. Proline and the non-protein amino acid γ-ABU[3] were consistently present at low levels (less than 1%). Between the flag leaf and ear, only glutamate and glutamine showed reasonably consistent differences. They showed an inverse relationship, with the mole percentage of glutamine increasing in the direction of transport and glutamate decreasing.

On a mole percentage basis, the main features of the endosperm cavity sap composition (Table II; also, see Table I) were generally similar to those of the sieve tube exudate supplying the grain. However, cysteine, virtually absent from sieve tube exudate, consistently accounted for 1 to 2% of the total amino acids in endosperm cavity sap. Alanine accounted for about 20%, a much higher proportion than in sieve tube sap (about 5%). Less notable differences appear for glutamate and proline (as for alanine and cysteine, they were relatively more prominent in the endosperm cavity) and for threonine, valine, and isoleucine (relatively more abundant in the sieve tube).

The molar ratio of carbon to nitrogen for the total amino acid fraction in both the sieve tube exudates and endosperm cavity sap samples was consistently close to 3.0 (a weight ratio of 2.6 mg C/mg N).

The more detailed analysis of gradients along the final portion of the sieve tube pathway in the ear and within the grain itself likewise showed little or no indication of composition differences along that part of the pathway (Table III). These samples were obtained from cv Gigas rather than SUN 9E (Tables I and II). However, aside from relatively more glutamine and less serine in the Gigas exudate, the amino acid compositions of Gigas and SUN 9E phloem exudates were quite similar.

DISCUSSION

Due to the small amounts of exudate obtainable, and to the labor involved in their collection and assay, replicate samples were not taken within an experiment. Nevertheless, the low variability of the analytical procedure, as evidenced in the data of Table I, together with the consistency of the major trends noted, allow confidence in the conclusions reached.

With the exceptions of asparagine, glutamate, glutamine, and glycine, the relative concentrations of the individual amino acids reported by Simpson and Dalling (23) for wheat flat leaf phloem exudate are in general agreement with our values for aphid stylet exudate. Those workers used EDTA to stimulate phloem exudation, and ignored the presence of substantial proportions of phenylalanine and tyrosine in calculating their results, a judgment supported by our finding of quite low proportions of those amino acids in stylet exudate. The fact that their exudate samples were collected from excised leaves of plants grown under nitrogen-limiting conditions may account for the differences noted, especially in glutamate and glutamine content. At the midpoint of grain filling (their 15-d samples), their ratio of glutamate to glutamine was about 7:1. This had nearly reversed, to about 1:3, by the later stages of grain filling. The increased preponderance of glutamine toward the end of grain filling has also been noted in stylet exudate collected from the peduncle (2).

Comparisons of phloem exudate from other cereals are avail-

[3] Abbreviation: γ-ABU, γ-aminobutyric acid.

Table I. *Stability of the Amino Acid Composition of Endosperm Cavity Sap and Peduncle Stylet Exudate Samples Subjected to Various Treatments after Collection*

After collection, the samples were divided under mineral oil into three (cavity sap) or two (stylet exudate) nearly equal volumes and treated in the manner indicated. Stylet exudate was obtained from a plant 28 d after anthesis, and endosperm cavity sap from a plant 21 d after anthesis.

Amino Acid	Endosperm Cavity Sap			Peduncle Stylet Exudate	
	0°C, 3 h	22°C, 3 h	Dry, 22°C, 3 d	Frozen	Dry, 22°C, 3 d
			mol %		
γ-ABU	0.4	0.5	0.5	0.3	0.3
Ala	19.4	20.4	18.5	2.4	3.2
Arg	0.9	1.0	0.9	0.1	0.2
Asn	3.5	3.8	3.7	3.3	3.3
Asp	1.1	1.0	1.1	1.3	1.6
Cys	1.9	1.4	1.6	<0.05	<0.05
Gln	43.1	43.2	45.8	51.5	49.9
Glu	1.5	1.5	1.7	2.4	2.6
Gly	2.7	2.6	2.7	1.2	1.3
His	0.8	0.5	0.8	1.4	1.4
Hse	0.2	<0.05	<0.05	<0.05	<0.05
Ile	1.0	1.0	0.9	3.4	3.5
Leu	1.0	1.2	1.0	3.6	3.5
Lys	2.8	2.8	2.8	2.7	2.6
Met	0.6	0.9	0.9	2.3	2.2
Phe	0.4	0.5	0.3	4.7	4.7
Pro	0.2	0.3	0.2	0.1	0.1
Ser	13.9	12.6	12.1	6.0	6.5
Thr	2.5	2.5	2.5	4.9	5.0
Trp	0.1	0.1	<0.1	0.5	0.6
Tyr	0.3	0.3	0.2	1.9	1.8
Val	2.0	1.9	1.9	6.0	5.6

Table II. *Amino Acid Compositions of Sieve Tube Saps Along the Pathway from the Flag Leaf to Developing Wheat Grains, and of Endosperm Cavity Sap Collected from the Grains*

In each case all of the samples were obtained from a single plant.

Amino Acid	21 d Post-Anthesis				25 d Post-Anthesis			
	Flag leaf	Peduncle	Grain pedicel	Endosperm cavity	Flag leaf	Peduncle	Grain pedicel	Endosperm cavity
				mol %				
γ-ABU	0.5	0.2	0.5	0.6	0.5	0.2	0.4	0.5
Ala	6.4	6.2	4.5	21.9	14.5	6.7	4.6	17.3
Arg	2.3	4.6	2.7	1.3	1.7	3.3	3.3	2.1
Asn	4.0	3.7	2.4	2.3	1.9	3.9	3.4	2.3
Asp	3.8	2.5	6.5	2.1	4.1	1.9	0.4	2.5
Cys	<0.05	<0.05	<0.05	1.3	<0.1	<0.1	<0.1	1.3
Gln	33.7	42.1	48.6	43.4	27.0	50.7	48.6	36.5
Glu	9.1	2.6	1.1	3.8	16.1	1.7	0.9	5.3
Gly	0.8	2.8	3.2	3.4	4.0	2.0	2.3	3.0
His	1.3	1.5	1.1	0.5	0.4	1.0	1.2	0.9
Ile	4.3	3.0	1.9	0.8	1.6	2.8	2.5	1.4
Leu	5.6	2.8	1.6	0.9	1.7	2.9	1.8	2.3
Lys	3.5	3.4	2.3	1.8	1.5	2.6	2.6	2.2
Met	0.1	<0.1	1.0	<0.1	<0.1	0.3	0.5	0.7
Phe	3.0	2.2	1.4	0.5	1.0	1.4	1.1	1.1
Pro	0.5	0.3	0.1	0.5	0.9	0.5	0.4	1.9
Ser	7.4	9.5	10.9	10.7	13.6	6.0	16.3	12.6
Thr	3.9	4.8	5.3	2.4	5.8	5.7	5.2	2.6
Trp	2.0	1.8	0.6	<0.1	<0.1	0.9	0.1	1.0
Tyr	1.5	1.1	0.8	0.3	0.9	1.0	0.8	0.6
Val	6.5	5.0	3.4	1.6	2.9	4.5	3.9	1.9

Table III. *Amino Acid Compositions of Phloem Exudates from the Rachis, Grain Pedicels, and Grain Vascular Bundle of cv Gigas (28 d Post-Anthesis)*

All samples were obtained from the same plant.

Amino Acid	Rachis	Grain Pedicel	Grain Vascular Bundle
		mol %	
γ-ABU	0.5	0.5	1.1
Ala	2.9	1.4	2.9
Arg	4.2	3.4	3.3
Asn	4.9	4.3	5.4
Asp	0.5	0.5	0.7
Cys	<0.05	<0.05	<0.05
Gln	66.2	68.9	63.9
Glu	1.3	0.6	0.3
Gly	1.7	0.9	1.3
His	1.3	1.0	1.1
Ile	1.4	1.9	2.3
Leu	0.7	1.2	1.6
Lys	2.4	1.8	2.1
Met	1.0	0.4	0.6
Phe	1.2	1.5	1.8
Pro	0.5	0.4	0.8
Ser	2.5	2.6	1.6
Thr	1.9	2.5	2.2
Trp	0.6	1.2	0.9
Tyr	0.6	0.7	0.7
Val	3.9	4.4	5.5

able only for rice (6, 7) and barley (26). In rice leaf phloem exudate, also collected from exuding stylets, asparagine and glutamine accounted for 30 to 40% of the amino acids, with asparagine always more prominent than glutamine. Alanine was substantially lower than in wheat exudate, and γ-ABU was not detected; the proportions of other amino acids were generally similar. Analysis of barley leaf phloem exudate, collected by EDTA-enhanced exudation (26), was less detailed. However, glutamine, glutamate, serine, alanine, and aspartate were the principle amino acids present, similar to their relative importance in our wheat leaf stylet exudate.

Useful comparisons may also be made between the amino acid composition of the assimilate stream supplying the grain and the total amino acid content (free amino acids plus protein) of the immature (1) and mature grain (12, 20). (Since there is a discontinuity in the xylem between the grain and the rachilla [30], the contribution of xylem transport should be negligible.) The high glutamine plus glutamate content of wheat phloem exudate is clearly reflected in the high percentage of the glutamate family of amino acids in the grain. The proportions of histidine, leucine, phenylalanine, tyrosine, and the sulfur amino acids appear to be somewhat higher in the grain, while serine and perhaps threonine are lower. The general impression gained from a comparison of the assimilate stream and grain composition is one of overall similarity, and this clearly applies to the proportions of total N and K in their dry matter (2). However, the relative contributions of phloem transport and subsequent metabolism to overall grain amino acid composition remain uncertain. Grains of cultured wheat ears grow normally with glutamine, asparagine, or NH_4NO_3 as sole nitrogen sources (19), and both the maternal tissues surrounding developing seeds (13, 14, 17, 19, 21) and the grain endosperm itself (11, 16) are capable of substantial amino acid interconversion.

Only minor changes in relative amino acid content appeared to occur along the sieve tube transport pathway from the flag leaf to the grain, although the extent to which the total amino acid content might change was not investigated. The only convincing change was in the increased amidation of glutamate. Other differences detected along the sieve tube pathway, while apparently real in a given experiment, were not consistent and may have arisen from variations in the amino acid content of different sieve tubes. The exuding stylets would have sampled only a few of the several hundred sieve tubes in a leaf or peduncle. Thus, differences between the flag leaves (Table II) may simply reflect differences in leaf condition, but variations between sieve tubes might be a contributing factor. Similarly, the composition of grain pedicel exudate may provide a better indication than that of stylet exudate of the overall assimilate supply to the developing grains.

The absence of changes in the amino acid composition of sieve tube and endosperm cavity sap samples, even when incubated for extended periods at room temperature, demonstrates the apparent inertness of these physiological fluids with respect to amino acid metabolism after their removal from contact with surrounding plant tissues, as found for other solutes and for total osmolality (4). Thus, the present observations further support the view that the endosperm cavity sap represents a basically inert metabolic compartment whose major properties are determined entirely by the balance of water and solute influx and efflux into and from surrounding tissues. This does not result in an unchanging composition *in vivo*, however, as a substantial range in the proportion of major solutes (sucrose, glucose, and total amino acids) has been observed (3). Only the total solute concentration remains fairly constant at 280 to 320 mosmolal (about 7.5 bars osmotic pressure), although this, too, has been observed to decrease appreciably in excised grains (DB Fisher, unpublished data).

Of the amino acids present in the endosperm cavity sap, alanine and cysteine showed the most pronounced differences from the composition of the sieve tube sap supplying the endosperm cavity compartment. The consistent presence of cysteine in endosperm cavity sap, coupled with its near absence from the sieve tube sap, indicates that it was transported in another form, perhaps as glutathione. The relatively high proportion of alanine in cavity sap may reflect a high transaminase activity in the crease tissues, such as was recently reported for the pedicel-placenta-chalazal region of corn (13).

While not determined directly in the present experiments, approximate concentration gradients for individual amino acids between the grain sieve tubes and the endosperm cavity may be calculated from data obtained from similar plants (2, 4, 5). Those experiments showed that amino acids accounted for about 25% of the sieve tube sap osmoticum which, when compared with the concentrations of total amino acids in the endosperm cavity sap (4, 5), indicates a concentration ratio (sieve tube sap/cavity sap) of about 2.3:1, with individual values (n = 16) ranging from 1.4:1 to 3.3:1. Since the mole percentages of individual amino acids in the crease sieve tube sap and endosperm cavity sap were similar, the relative concentrations of most amino acids in the two would likewise be about 2.3:1. Alanine and glutamate, however, are notable exceptions. Here (and for potassium [4]), transport appears to be occurring with little or no overall concentration gradient or, especially in the case of alanine, perhaps even against an overall gradient. Since these three solutes also differ in electrical charge (cavity sap pH is about 6.5 [8]), their movement across the crease tissues likewise appears to be independent of any overall electric potential difference between the sieve tube and endosperm cavity. The possibility of metabolic interconversions complicates the picture for alanine and glutamate. However, aside from the clearer example of potassium, there is, in general, a lack of discrimination for or against the transport of other anionic or cationic amino acids, indicating that movement from the sieve tube to endosperm cavity does not occur simply by free diffusion but, at least at some point

along the pathway, must be metabolically dependent. A similar conclusion has been drawn from the apparent lack of response of grain filling rate to changes in the sieve tube-endosperm cavity sap concentration gradients or to changes in endosperm cavity sap sucrose concentrations (5). Metabolically dependent "phloem unloading" has been demonstrated for *Vicia* stems parasitized by *Cuscuta* (29) and for legume seed coats (reviewed by Thorne [25]). In the case of *Cuscuta*, this appears to apply to the movement of solutes from the sieve tubes themselves (29). However, since the pathway for solute movement into developing seeds includes the parenchyma tissues of the crease (wheat) or the seed coat (legumes), the sites of metabolic dependence are as yet uncertain in those systems.

Acknowledgments—We thank Roger Gifford and Ian Wardlaw for their interest, advice and assistance in many ways during the course of this work, and Katie Helms for providing the aphid colonies.

LITERATURE CITED

1. DONOVAN GR, JW LEE 1978 Effect of the nitrogen source on grain development in detached wheat heads in liquid culture. Aust J Plant Physiol 5: 81–87
2. FISHER DB 1987 Changes in the concentration and composition of peduncle sieve tube sap during grain filling in normal and phosphate-deficient wheat plants. Aust J Plant Physiol. In press
3. FISHER DB, JM FRAME 1984 A guide to the use of the exuding stylet technique in phloem physiology. Planta 161: 385–393
4. FISHER DB, RM GIFFORD 1986 Accumulation and conversion of sugars by developing wheat grains. VI. Gradients along the transport pathway from the peduncle to the endosperm cavity during grain filling. Plant Physiol 82: 1024–1030
5. FISHER DB, RM GIFFORD 1987 Accumulation and conversion of sugars by developing wheat grains. VII. Effects of changes in sieve tube and endosperm cavity sap concentration on the grain filling rate. Plant Physiol. In press
6. FUKUMORITA T, M CHINO 1982 Sugar, amino acid and inorganic contents in rice phloem sap. Plant Cell Physiol 23: 273–283
7. FUKUMORITA T, Y NOZIRI, H HARAGAUCHI, M CHINO 1983 Inorganic content in rice phloem sap. Soil Sci Plant Nutr 29: 185–192
8. HO LC, RM GIFFORD 1984 Accumulation and conversion of sugars by developing wheat grains. V. The endosperm apoplast and apoplastic transport. J Exptl Bot 35: 58–73
9. JENNER CF 1974 Factors in the grain regulating the accumulation of starch. R Soc NZ Bull 12: 901–908
10. KAWABE S, T FUKUMORITA, M CHINO 1980 Collection of rice phloem sap from stylets from homopterous insects severed by YAG laser. Plant Cell Physiol 21: 1319–1327
11. KOLDERUP F 1979 Interconversion of amino acids in maturing wheat grain. *In* FAO/IEA Symposium on Seed Protein Improvement in Cereals and Legumes, Vol I. International Atomic Energy Agency, Vienna, pp 187–202
12. KHAN MK, BO EGGUM 1978 Effect of baking on the nutritive value of Pakistani bread. J Sci Food Agric 29: 1069–1075
13. LYZNIK LA, A RAFALSKI, K RACZYNSKA-BOJANOWSKA 1985 Amino acid metabolism in the pedicel-placenta-chalazal region of the developing maize kernel. Phytochemistry 24: 425–430
14. LYZNIK LA, W ZDROJEWSKI, M NEUMANN, J MACEWICZ, K RACZYNSKA-BAJANOWSKA 1982 A possible role of pedicel-placental-chalazal tissues in the amino acid supply to the developing maize endosperm. Maydica 27: 191–198
15. MACNICOL PK 1983 Developmental changes in the free amino acid pool and total protein amino acids of pea cotyledons (*Pisum sativum* L.). Plant Physiol 72: 492–497
16. MISRA S, A OAKS 1985 Glutamine metabolism in corn kernels cultured *in vitro*. Plant Physiol 77: 520–523
17. MURRAY DR, IR KENNEDY 1980 Changes in activities of enzymes of nitrogen metabolism in seedcoats and cotyledons during embryo development in pea seeds. Plant Physiol 66: 782–786
18. PATE JS 1983 Distribution of metabolites. *In* FC Steward, RGS Bidwell, eds, Plant Physiology, A Treatise, Vol 8. Academic Press, Orlando, FL, pp 335–401
19. PEOPLES MB, CA ATKINS, JS PATE, DR MURRAY 1985 Nitrogen nutrition and metabolic interconversion of nitrogenous solutes in developing cowpea fruits. Plant Physiol 77: 382–388
20. POMERANZ Y, KF FINNEY, RC HOSENEY 1966 Amino-acid composition of maturing wheat. J Sci Food Agric 17: 485–487
21. PORTER GA, DP KNIEVEL, JC SHANNON 1985 Sugar efflux from maize (*Zea mays* L.) pedicel tissue. Plant Physiol 77: 524–531
22. RADLEY M 1978 Factors affecting grain enlargement in wheat. J Exptl Bot 29: 919–934
23. SIMPSON RJ, MJ DALLING 1981 Nitrogen redistribution during grain growth in wheat (*Triticum aestivum* L.). III. Enzymology and transport of amino acids from senescing flag leaves. Planta 151: 447–456
24. TAPUHI Y, DE SCHMITT, W LINDNER, BL KARGER 1981 Dansylation of amino acids for high-performance liquid chromatography analysis. Anal Biochem 115: 123–129
25. THORNE JH 1985 Phloem unloading of C and N assimilates in developing seeds. Annu Rev Plant Physiol 36: 317–343
26. TULLY RE, AD HANSON 1979 Amino acids translocated from turgid and water-stressed barley leaves. I. Phloem exudation studies. Plant Physiol 64: 460–466
27. VAN DIE J 1974 The developing fruits of *Cocos nucifera* and *Phoenix dactylifera* as physiological sinks importing and assimilating the mobile aqueous phase of the sieve tube system. Acta Bot Neerl 23: 521–541
28. WIERSUM LK 1967 The mass-flow theory of phloem transport; a supporting calculation. J Exptl Bot 18: 160–162
29. WOLSWINKEL P 1974 Enhanced rate of ^{14}C-solute release to the free space by the phloem of *Vicia faba* stems parasitized by Cuscuta species. Acta Bot Neerl 23: 177–188
30. ZEE S-Y, TP O'BRIEN 1970 A special type of tracheary element associated with xylem discontinuity in the floral axis of wheat. Aust J Biol Sci 23: 783–791