

Short Communication

Free Amino Acid Composition of Phloem Sap and Growing Fruit of *Lycopersicon esculentum*

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Pure phloem sap of tomato leaves was collected by stylectomy. Glutamine and glutamate were the predominant free amino acids translocated by the phloem stream. In developing fruits glutamine content increased significantly, reaching 35% of the total free amino acids. Comparison in the amino acid composition between the two tissues are discussed.

Key words: Amino acid — GABA — Glutamine — *Lycopersicon esculentum* — Phloem sap — Tomato fruit.

The growth of tomato fruit goes through different phases. The early phase is characterized by high metabolic activity and rapid cell division of the tissue; at a later developmental phase, the cells expand, and seeds are formed (Gillaspy et al. 1993). As sink organs, fruits are dependent on the translocation of sucrose, amino acids, and organic acids to their cells. The rate of import of these photoassimilates from the leaves is governed by the metabolic activity of the fruit (Ho 1988). In the case of the tomato, green fruit cells contain photosynthetically active chloroplasts which give the developing fruit its green appearance, although photosynthetic carbon dioxide fixation in the fruit chloroplasts makes only a minor contribution to fruit growth (Piechulla et al. 1987). Therefore, developing tomato fruit depends on the delivery of photoassimilates from the leaves.

Amino acids are the main transport form for nitrogen, and their composition pattern in the phloem sap is specific for each plant species (Lohaus 1995). Analysis of nitrogen compounds were previously reported in xylem, petiole and leaf exudates of tomato plants, all of them collected by bleeding procedures (Gao et al. 1996, Walker and Ho 1977, Lorenz 1976). However, the precise content of N-forms of pure phloem sap of tomato plants has not been previously determined.

In the present investigation, phloem sap of tomato plants was obtained by the aphid stylet technique (Barlow

and McCully 1972) and the EDTA-enhanced exudation (King and Zeevaart 1974). In a comparative study of the free amino acid composition of these phloem samples, we found that glutamine and glutamate were the predominant N-forms in the phloem translocation stream. The level of free amino acids in developing fruits was also examined.

Plants used in these experiments (*Lycopersicon esculentum* cv. Platense) were grown in the greenhouses of Göttingen University from January to August. Each pot was watered once every two weeks with 0.2% Wuxal nutrient solution (Aglukon) containing 11 mM N and watered on other days with tap water. Phloem sap was obtained simultaneously from fully expanded leaves of thirty-day-old plants using two different techniques. The leaf of the plant was illuminated at $400 \mu\text{mol m}^{-2} \text{s}^{-1}$ during collection. Phloem exudates were collected according to Weibull et al. (1990) with the modification of cutting the leaf and immersing it immediately in 6 ml of 20 mM EDTA (pH 9). Hourly samples were taken and kept frozen until analysis. Pure sieve tube sap was obtained by stylectomy from the minor veins of tomato leaves and collected in a micro capillary using a micromanipulator. Aphids (*Myzus persicae*) were collected in the greenhouses and put on leaves approximately two h before shooting them with a laser beam. It took about one h to obtain 30 to 50 nl of phloem sap, which was kept under liquid nitrogen until analysis. The phloem exudates from leaf cuts or severed aphid stylets were collected in the same illuminated chamber at 90% relative humidity and 25°C.

Leaf and pericarp tissue were extracted according to Winter et al. (1992), with modifications. The tissue (1 to 2 g) was ground in a mortar to a fine powder while kept under liquid nitrogen. To this material, 0.6 ml of a buffer containing 20 mM HEPES (pH 7.0), 5 mM EDTA, 10 mM NaF, and 5 ml chloroform/methanol (1.5/3.5, v/v) were added. The sample was homogenized until it was completely thawed and then kept on ice for 30 min. From this homogenate, the water-soluble amino acids were extracted twice with 3 ml water. The aqueous phases were combined and evaporated in a rotary evaporator. The dried residue was dissolved in 2 ml HPLC water (Merk) and stored at -80°C until analysis. The amino acid composition was de-

Abbreviation: GABA, γ -aminobutyric acid.

terminated by derivatisation with ninhydrine or *o*-phthalaldehyde and HPLC according to Riens et al. (1991).

Translocation of free amino acids through the phloem sap—To determine the free amino acid composition of tomato phloem sap, we used the aphid stylet technique in combination with a laser beam to sever the aphid stylet after it attached to the leaf (Kawabe et al. 1980). The hairy leaves presented a major obstacle. At least ten trials per sample were needed to obtain a measurable amount of phloem sap. Because of these difficulties, we also made a comparative study of two collecting methods (Weibull et al. 1990, Girousse et al. 1991) by obtaining phloem sap as exudate from the cut plant parts. This phloem exudate was collected by inserting the cut end of a leaf petiole into an EDTA solution (King and Zeevaart 1974); samples were taken while the leaf was being illuminated. Analysis of the relative free amino acid content in these samples was performed by HPLC and fluorescence detection of the derivatisation products. The results are shown in Table 1. The greatest difference between the relative amino acid composition in the phloem sap obtained by the two methods was the higher molar glutamine content of the phloem sap obtained from stylectomy. It is possible that the low glutamine content in the exudate from cut leaves may be due to glutaminase activity released by wounding. Minor differences between the two methods were observed in the mo-

lar contents of GABA, asparagine, leucine, and phenylalanine. Thus, the results obtained by these two methods indicate that glutamine and glutamate are the most abundant free amino acids present in the phloem sap of tomato plants.

Free amino acid composition of developing fruit—The most pronounced variations in individual amino acid during fruit development were in the relative molar contents of glutamate, glutamine, and GABA (Fig. 1). The glutamine molar content was low in the early developmental phase of the fruit, and its increase during fruit growth was accompanied by a decrease in the glutamate content (Fig. 1A–B). In the immature fruits, a notably high content of GABA was observed (Fig. 1C), which diminished when the fruits enlarged. Minor changes were observed in the relative molar content of proline, which decreased from 9 to 6%, as the fruits increased their weight (data not shown). Changes in glutamine and glutamate content of fruits at different growth stages were evident, although comparison with their content in the leaf phloem sap was difficult. Analysis of the pure phloem sap of peduncles and pedicel at several fruit developmental stages would also be necessary to correlate these variations with the free amino acid content of the transport system.

For many plants the aphid technique of Barlow and McCully (1972) is the most reliable way to obtain pure

Table 1 Comparison of the relative molar content of amino acids in phloem sap obtained by stylet- or EDTA-enhanced exudation technique of *Lycopersicon esculentum* cv. Platense leaves

Amino acid	Relative content (mol %)		
	Stylet exudate	EDTA-enhanced exudate	Leaf
Aspartate	7.6±0.5	9.1±0.6	14.0±1.6
Glutamate	15.7±1.6	15.8±1.6	32.1±4.0
Asparagine	1.9±0.8	6.3±1.5	6.9±1.5
Serine	5.0±0.3	6.9±0.4	3.4±0.6
Glutamine	30.4±8.0	16.5±3.3	6.3±1.3
Glycine	0.9±0.0	2.8±0.1	2.4±0.6
Threonine	8.7±0.6	7.3±0.5	9.5±1.5
Alanine	4.9±0.4	3.3±0.3	6.3±0.6
GABA	2.9±0.5	9.7±1.7	4.2±0.7
Valine	3.5±0.2	3.2±0.2	1.6±0.2
Isoleucine	2.2±0.3	2.7±0.5	1.2±0.2
Phenylalanine	7.2±0.7	3.0±0.5	2.3±0.7
Leucine	2.6±0.3	4.4±0.5	1.3±0.1
Lysine	2.8±0.4	2.5±0.2	0.9±0.1
Others	3.7±0.8	6.5±1.0	7.6±1.5

Thirty-day-old plants were used for the experiment. Samples of stylet- and EDTA-enhanced exudates were collected from 1 to 2 h under identical conditions of temperature, humidity, and illumination as described in Materials and Methods. Total free amino acid concentration of pure phloem sap (stylet exudate) was 395±49 mM. Results are means±SD of three replicates from different plants.

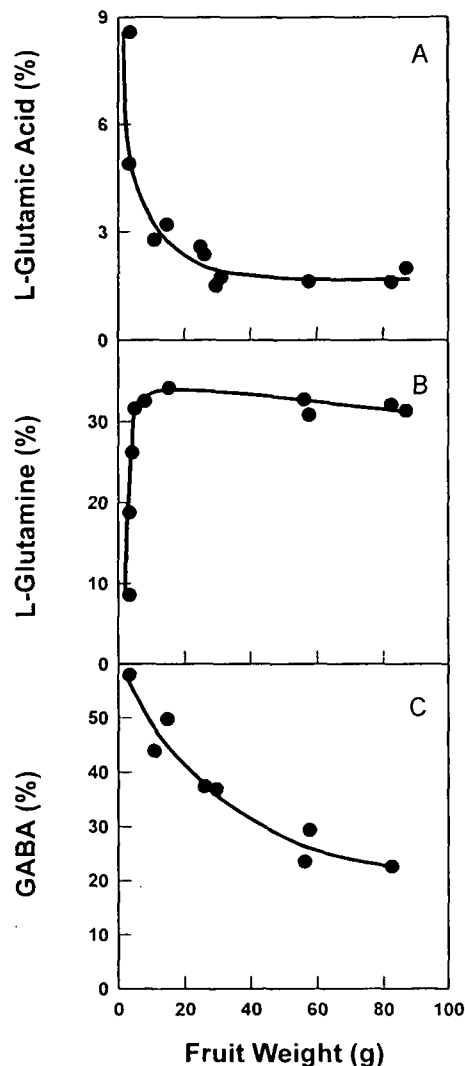


Fig. 1 Relative molar content (%) of L-glutamic acid (A), L-glutamine (B), and γ -aminobutyric acid (C) in developing *Lycopersicon esculentum* cv. Platense fruits. These fruits were all immature green around 1 to 5 cm in their larger diameter. Fruits were harvested until 35 d after anthesis followed by fresh weight determination (X-axis). Free amino acids content was assayed in the pericarp of every fruit. Each point represents the mean of three determinations.

phloem sap samples, but it is difficult to use this method with hairy plants (Lohaus 1995). Comparative studies of two phloem sap collecting methods were already done in oat, barley, and alfalfa (Weibull et al. 1990, Girousse et al. 1991). By using stylectomy and EDTA-enhanced phloem exudation, we were able to determine the free amino acid composition of the phloem sap of tomato leaves. Glutamine and glutamate are the main amino acids translocated by the phloem, followed by aspartate, threonine, and serine. Walker and Ho (1977) identified aspartate and gluta-

mate as the only amino acids present in the petiole after feeding the tomato leaf with $^{14}\text{CO}_2$, although glycine, proline, valine, threonine, alanine, and leucine were found in the pedicel. They detected no glutamine whatsoever. It may be noted that the glutamine contents of the leaf and bleeding sap are largely dependent on the nitrogen supply of the growing plant (Martinez et al. 1994, Lorenz 1976). Our results clearly indicate that glutamine and glutamate are the major free amino acids transported by the phloem system of tomato.

The relatively low GABA content (2.9%) of the stylet exudate indicates that this amino acid contributes little to the transport of organic nitrogen in the phloem. Similar results have been obtained with rice (Fukumorita and Chino 1982), wheat (Hayashi and Chino 1986) and alfalfa (Girousse et al. 1991). The relatively higher content of GABA (9.7%) in the EDTA-enhanced exudate than in the stylet exudate is probably caused by a wounding response to cutting the leaf. Comparable observations were reported by Girousse et al. (1991). The metabolic role of GABA in higher plants is still under debate (Satya Narayan and Nair 1990, Bown and Shelp 1997).

Fruit development and growth are dependent on the translocation of photoassimilates to the fruit cells (Ho 1988). At present, the physiological factors controlling the metabolic activities that influence fruit growth are not well understood. Much effort has been devoted to the study of sink strength during later phases of fruit development with respect to sucrose metabolism (Guan and Janes 1991) and accumulation (Yelle et al. 1991, Wang et al. 1993), but little is known about amino acid translocation, uptake and metabolism during fruit development. In their early developmental phase, most fruits can be classified as utilization sinks because of their high metabolic activities (Ho 1988). For such fruits, it was proposed that the sucrose uptake occurs by simple diffusion (Ruan and Patrick 1995). In this paper, we show an increase in glutamine content during early fruit development, probably due to glutamine synthesis in the pericarp and/or import via the phloem sap. Although the comparison between the content of photoassimilates in the phloem sap and fruit is difficult, we hypothesize that uptake in the pericarp of fruits is coupled to the mass flow of sucrose, as proposed by Winter et al. (1992). Another characteristic of the immature fruits was also the relatively high GABA content, which may play a role in the resistance of the fruits to pathogenic agents (Cohen et al. 1994, Sherf and Kolattukudy 1993). Experiments are currently in progress to determine the capacity of growing tomato fruits to synthesize GABA and glutamine.

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