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Short Communication

Collection and Chemical Composition of Pure Phloem Sap from *Zea mays* L.

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Pure phloem sap was collected from leaf sheaths of *Zea mays* L. by the insect laser technique, and its chemical composition was analyzed. Sucrose was the only sugar detected. The predominant inorganic ions were K^+ and Cl^-. The adenylate energy charge of phloem sap was between 0.72 and 0.86.

Key words: Amino acid — Nucleotide — Phloem sap — Planthopper — Sugar — *Zea mays* L.

Many obscure points remain to be resolved with respect to the transport of photosynthates from source to sink through sieve tubes. It is very important to be able to collect and analyze the phloem sap if an attempt is to be made to explain the phloem transport system. The insect laser technique (Kawabe et al. 1980) has been shown to be useful method for the study of this problem. The insect laser technique has been used for detailed determination of the chemical composition of phloem sap from C_3 plants, such as wheat and rice (Fukumorita and Chino 1982, Hayashi and Chino 1986). Fisher and Frame (1984) collected phloem sap from *Zea mays* L. using an aphid technique, but its chemical composition has not been reported. The present report provides the first description of the chemical composition of pure phloem sap from maize. In addition, the concentration of nucleotides in phloem sap has been precisely determined by HPLC, and the adenylate energy charge has been calculated.

The maize plants were grown under natural light conditions in a greenhouse at 25°C, with a supply of a complete nutrient solution that contained 0.75 mM $NaNO_3$, 0.38 mM NH_4NO_3, 0.07 mM KH_2PO_4, 0.48 mM K_2SO_4, 0.58 mM $CaCl_2$, 0.04 mM EDTA-NaFe, 0.50 mM H_3BO_3, 0.004 mM $MnSO_4$, 0.004 mM $ZnSO_4$, 0.001 mM $CuSO_4$, 0.001 mM Na_2MoO_4, and 0.42 mM $MgSO_4$. The solution was renewed every 3 days, and the pH was adjusted to 5.5 daily.

Phloem sap was collected from the 4th or 6th leaf sheath of maize plants at the 6th to 8th leaf stage in a collecting room (25,000 lux at plant height, 25°C) by the insect laser technique (Kawabe et al. 1980). Small brown planthoppers (*Loadephax striatellus* Fallen) were used and the stylets of insects were cut by a YAG laser beam. The exudate was easily collected by placing microcapillaries (Drummond Scientific Co., Broomall, Penn., U.S.A.) of 1 μl or 0.5 μl capacity over the cut end of stylets with a micromanipulator. Collected phloem sap was diluted with 99 μl of distilled water and stored in a freezer at −20°C until analysis. The amount of phloem sap collected from one stylet was between 0.09 and 1.86 μl. The rate of exudation of maize phloem sap was generally lower than that of rice and wheat.

Anions and cations were analyzed with an ion-chromatoanalyzer (Model IC-100, Yokogawa Electric Co., Tokyo, Japan). Other compounds, such as amino acids, nucleotides and sugars, were determined by HPLC (Model L-6200, Hitachi Co., Tokyo, Japan). The cations, anions and amino acid were analyzed under the same conditions as those described previously (Hayashi and Chino 1986). The conditions for the analysis of sugars by HPLC were as follows; column, Gelpack GL-C610 (Hitachi-kasei Co., Tokyo, Japan); column temperature, 60°C; mobile phase, H_2O; flow rate, 1.0 ml/min; detector, refractive index monitor (Model 655A-30, Hitachi Ltd.). The nucleotides were determined by HPLC as described by Nieman and Clark (1984). The collection of phloem sap from maize was found to be more difficult than that from rice and wheat because the insects scarcely fed on the maize plants. All the data shown in the Tables are from the analysis of representative samples of phloem sap which were exuded from 6th leaf sheath of maize at the stage of the 8.4th leaf during the course of about 4 h under 25,000 lux at 25°C, in the collecting room. The rate of exudation of the sap was 0.07 μl/h.

Table 1 shows the concentrations of each of the solu-

Table 1 Chemical composition of maize phloem sap

Component	Concentration	
	(mM)	(%)
K^+	497.5	21.2
Na^+	182.0	7.7
NH_4^+	85.3	3.6
Cl^-	273.0	11.6
PO_4^{3-}	33.0	1.4
NO_3^-	3.2	0.1
SO_4^{2-}	2.4	0.1
Sucrose	900.4	38.3
Total amino acids	375.2	16.0
Total nucleotides	3.7	0.2

ble components determined in the maize phloem sap. The concentration of total soluble components was about four times that obtained by plasmolyzing sieve-tube membranes and companion cells of maize (Evert et al. 1978) and it was much higher than that of rice (Fukumorita and Chino 1982) or wheat (Hayashi and Chino 1986). Thus, the low rate of exudation is possibly the reason for the concentration of phloem sap from maize plant at the time of sampling. The predominant inorganic ions were K^+ and Cl^-. The relative levels of Na^+, NH_4^+ and Cl^- were much higher than those in rice and wheat. The only sugar present in maize phloem sap was sucrose and the amount of sugar, as a proportion of the total soluble material, was higher than that in rice and wheat.

The amount of amino acids, as a portion of the total soluble material, was much lower than that in rice and wheat. Table 2 shows the amino acid contents of the maize phloem sap. About 80% of the total amino acids consisted of Asp, Thr, Ser, Glu, Gly, Ala, Val, Ile and Lys. The proportion of Asp in the total amino acid pool in maize phloem sap was one fourth of that in wheat phloem sap (Hayashi and Chino 1986). The relative amount of Glu was lower, but relative amounts of Ser, Gly and Val were higher than those in wheat phloem sap.

The composition in terms of nucleotides is shown in Table 3. Over 40% of the nucleotide pool was taken up by ATP. ATP, ADP and AMP made up about 80% of the total pool of nucleotides. Since sieve tubes contain few mitochondria, most of the ATP in the sap is possibly supplied by companion cells which have many mitochondria. Pavlinova and Afanasjeva (1962) found ATP, ADP, and AMP in the complete bundles of sugar beet. Kluge and Ziegler (1964), Gardner and Peel (1969, 1972), Hall and Baker (1972) and Kluge et al. (1970) found high concentrations of ATP in the phloem exudate of many kinds of plant. The energy charge of the maize sap was found to be between 0.72 and 0.86. These values are similar to the values of 0.88 for *Robinia* sap (Kluge and Ziegler 1964) and 0.77 for *Tilia* sap (Ziegler and Kluge 1962). These results appear to support the hypothesis that the mechanism of sucrose loading in sieve tubes is energy dependent.

Table 2 Amino acid contents in maize phloem sap

Amino acid	Concentration	
	(mM)	(%)
Aspartic acid	17.3	4.6
Threonine	14.3	3.8
Serine	27.8	7.4
Asparagine	7.2	1.9
Glutamic acid	59.0	15.7
Glutamine	0.0	0.0
Glycine	82.2	21.9
Alanine	5.0	1.3
Cysteine	0.0	0.0
Methionine	40.1	10.7
Isoleucine	19.9	5.3
Leucine	23.7	6.3
Tyrosine	8.6	2.3
Phenylalanine	9.0	2.4
γ-amino butyric acid	0.0	0.0
Lysine	23.2	6.2
Histidine	4.4	1.2
Arginine	9.4	2.5
Total concentration	375.2	100.0

As described above for maize, a C_4 plant, the relative level of sucrose was higher than that in C_3 plants such as

Table 3 Nucleotide content of the maize phloem sap

Nucleotide	Concentration	
	(mM)	(%)
CMP	0.00	0.0
UMP	0.06	1.6
AMP	0.26	7.0
IMP	0.00	0.0
GMP	0.00	0.0
CDP	0.00	0.0
UDP	0.14	3.8
ADP	0.82	22.1
GDP	0.17	4.6
CTP	0.00	0.0
UTP	0.42	11.3
ATP	1.52	41.0
GTP	0.32	8.6
Total concentration	3.71	100.0
Adenylate energy charge	0.74	

rice and wheat. The proportion of amino acids was very much lower than in C_3 plants and the relative amounts of Ser, Val and Gly were higher and those of Asp and Glu were lower, when considered as a percentage of the total pool of amino acids. It is necessary now to collect and analyze the phloem sap from maize at various leaf stages and under various environmental conditions. Further investigations are also needed of various C_4 plants to confirm whether or not the results obtained here are characteristic of the composition of phloem sap of C_4 plants.

Only a little detailed information is available to date about the concentration of the various nucleotides in pure phloem sap, with the exception of the data obtained by Hayashi and Chino (1990). The nucleotide concentrations obtained in the present study are similar to those obtained with rice by Hayashi and Chino (1990).

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