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Effect of Potassium Supply on the Rate of Phloem Sap Exudation and the Composition of Phloem Sap of *Ricinus communis*

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ABSTRACT

The composition of phloem sap has been investigated in *Ricinus communis* var. gibsonii, grown for 2 weeks on nutrient solution of low and high potassium content (K_1 and K_2). Diagonal cuts were made in the bark of the stem resulting in the exudation of clear droplets which mainly consisted of phloem sap. Although the plants at low K (0.4 mM) and high K (1 mM) did not differ in growth, leaf area, height, or stem circumference, the rate of exudation of the high K plants was about twice as high as that of the plants with the lower K supply. This promoting effect of K on exudation did not result in a dilution of organic (sucrose, UDP-glucose, ATP, UTP) and inorganic constituents of the phloem sap. For the following compounds, even significantly higher concentrations in the exudate were observed in the K_2 plants: potassium, raffinose, glucose 6-phosphate, and fructose 6-phosphate. Also, the osmotic pressure of the phloem sap was substantially increased in the higher K treatment. Experiments in which labeled $^{14}CO_2$ was applied to one leaf showed that K had a favorable effect on the assimilation of CO_2, and in particular promoted the export of photosynthates from the leaf. It is suggested that the higher rate of phloem-loading in the plants with the better K supply is due to the higher CO_2 assimilation rate and especially to a better provision of ATP required for phloem loading. Higher phloem-loading rates result in higher osmotic pressure in the sieve tubes which probably gave rise to the higher flow rates observed in the plants with improved K supply.

Various papers published during recent years have shown that K affects the translocation rate of photosynthates (1, 5, 9, 14) for reasons not yet understood. It is known that K favors photophosphorylation (15), and it is supposed that higher ATP levels have an impact on phloem-loading (8). The object of this investigation was to examine whether K has an influence on the composition of the phloem sap because it was assumed that an effect of K on phloem-loading should be reflected by the concentration of photosynthates dissolved in the phloem sap.

MATERIALS AND METHODS

Castor oil plants (*Ricinus communis* var. gibsonii) were grown in nutrient solution in a growth chamber under the following conditions: light intensity, 12,000 lux; relative humidity, 70 to 80%; day temperature, 27 C; night temperature, 19 C; day length, 16 hr. Solution tanks of 7-liter capacity each contained two plants. Nutrient solutions were discarded and replaced with fresh solution at weekly intervals.

For the first 4 weeks, all plants were grown in a uniform solution of the following composition in mM: NH_4NO_3, 2; $Ca(NO_3)_2$, 2; NaH_2PO_4, 1; K_2SO_4, 1; $MgSO_4$, 2; $CaCl_2$, 3; in μM: Fe chelate, 20; $MnSO_4$, 14; H_3BO_3, 10; $CuSO_4$, 1.4; $ZnSO_4$, 1.4; $Na_6Mo_7O_{24}$, 0.3.

After the initial 4 weeks, a K-free solution which contained all of the other nutrients at the concentrations indicated above was applied for a further week after which differential K treatments were applied with concentrations of 0.4 (K_1) and 1 meq K/l (K_2). To obtain as many samples as possible, castor oil plants were grown continually under these experimental conditions. The plants thus obtained did not differ significantly in growth, plant height, leaf development, or leaf area between treatments.

After 2 weeks of differential K nutrition, droplets of phloem sap were collected from the plants using a capillary. Sap collection always took place 6 hr after the start of assimilation, between 10 and 13 hr. For this, diagonal cuts of 5 mm in length were made with a razor blade in the bark of the stem midway between the base of the stem and the lowest petiole (at about the fifth internode from the base). The plants were cut three times at the most, then discarded. The technique of sap collection has been described in detail by Hall *et al.* (7).

In the main experiments, exudation rate as well as the contents of different constituents of phloem sap were measured. The exudation rate was determined with individual plants by measuring the amount of sap exuded every 6 min during 3 hr. For the determination of sap constituents or osmotic pressure, however, sets of four plants each of either treatment were taken. The droplets exuded by these four plants were pooled in a tube and stored in an ice bath.

In a further experiment, the rate of translocation of labeled photosynthates from the leaf to the phloem sap was studied. An almost fully developed leaf of the plant was first supplied with $^{14}CO_2$ (1 μCi) for 10 min under strictly controlled conditions and thereafter kept in normal atmosphere for an additional 20 min. Phloem sap was collected from each individual plant for 3 hr according to the method described above. The ^{14}C activity of the phloem sap and of the $^{14}CO_2$-treated leaf was measured using a liquid scintillation counter.

A second additional experiment was to study whether the exudate was in fact phloem sap. In this, Ca was used as an indicator since this element is only slightly "phloem-mobile." In addition to the nutrients indicated above, the plants received 0.1 mCi $^{45}CaCl_2$ in the nutrient solution. After treatment with ^{45}Ca for 6 hr exudate was tapped during 1 hr. Then the plants were decapitated and the xylem sap exuded for the remaining stem was collected. The ^{45}Ca label of phloem and xylem sap, and of the youngest leaf was measured in the liquid scintillation counter.

The following values from the main experiments were determined directly: (*a*) the osmotic pressure of the phloem sap by microcryoscopy (18); (*b*) the cations K, Na, Mg, and Ca by atomic absorption spectrophotometry; (*c*) the anions chloride potentiometrically using the chloride titrator and phosphate col-

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orimetrically according to the vanadate molybdate method (12); (*d*) amino acids by two-dimensional paper chromatography and staining with ninhydrin (13).

To determine the contents of coenzymes, malate, and glucose-6-P, an aliquot amount of 1.2 M $HClO_4$ was added to the cool sap for protein precipitation, and thereafter the sap was neutralized with 3 M KOH. The precipitate was separated by centrifugation at 3,000 rpm. In the protein-free sap, the above components could be determined enzymically according to Bergmeyer (3).

Since acid protein precipitation gives rise to hydrolysis of sucrose, 0.12 ml K hexacyano-ferrate (85 mM), 0.12 ml zinc sulfate (250 mM), and 0.25 ml NaOH (0.1 N)/ml sap were added for protein precipitation in case the sugars in the phloem sap were to be analyzed. The contents of glucose, fructose, sucrose, raffinose, and fructose-6-P were also determined enzymically (3). The analyses were repeated several times, depending on the accuracy desired and the difficulties involved, with fresh sap from experimental sets of 16 plants each.

The statistical evaluation refers to plants of different sets of each treatment. Significant differences are indicated in the tables by asterisks (* = $P < 5\%$; ** = $P < 1\%$; *** = $P < 0.1\%$).

RESULTS

As can be seen in Table I, the content of labeled Ca in phloem exudate was low compared with that of xylem sap and of leaves. Since phloem sap is known to be very low in Ca, it is concluded that the exudate obtained consisted mainly of phloem sap.

There was a substantial difference in the exudation rates between the K treatments. Figure 1 represents the cumulative exudation volumes during an experimental period of 3 hr. Each point of the curves is a mean value of six individual samples. Measurements were carried out at intervals of 6 min. The total quantity of exudate at the end of the experiment was 2.49 ml in the K_2 and 1.35 ml in the K_1 plants. This difference is highly significant. The higher exudation rate did not result in a dilution of the sap. Table II shows that there was no significant difference in concentrations of inorganic constituents, with the exception of K^+, the concentration of which was considerably higher in the K_2 treatment. The osmotic potential of the sap was significantly increased in the plants with the higher K supply.

Organic constituents of the phloem sap are listed in Table III. The sucrose level was hardly affected by K. There was a clear effect of K on the concentration of fructose-6-P, glucose-6-P, and raffinose, and some amino acids (Table IV). The concentrations of glutamic acid, aspartic acid, and leucine were higher and the concentrations of glutamine and serine were significantly lower in the K_2 plants than in the K_1 plants. As can be seen, glutamine was by far the dominant amino acid. The concentration of malate in the exudate appears to be rather low. There was, however, a significant K effect. All concentrations presented in Tables II, III, and IV are average values over a period of 3 hr during which all of the collected exudates were pooled. According to this procedure, no information can be given whether the concentration of the exudate changed during this experimental period.

The results obtained from the experiment, in which labeled CO_2 was applied to one leaf, show a similar effect. The exudation rate was significantly increased by K, whereas the concentration of ^{14}C-labeled solutes in the sap was hardly affected. The total amount of labeled material exuded by the plants was considerably increased in the K_2 plants due to the higher exudation rate (Table V). The remaining ^{14}C label in the treated leaf 210 min after ^{14}C application did not differ much between treatments.

Table I. Content of ^{45}Ca in the Youngest Leaf, in Xylem Sap and Phloem Exudate

Values are means of two samples.

	K Treatment	
	0.4 meq/l	1.0 meq/l
Leaf, nCi/g dry wt	13.1	10.8
Xylem sap, nCi/ml	17.8	9.4
Phloem Exudate, nCi/ml	0.53	0.96

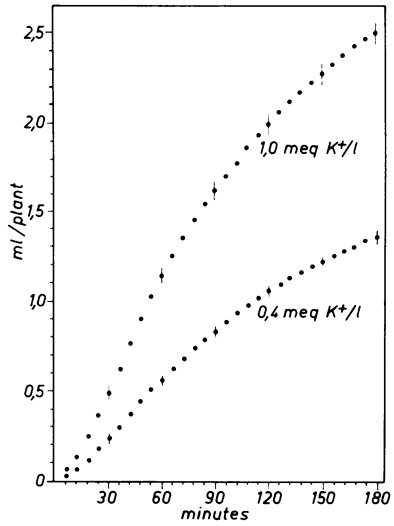

FIG. 1. Cumulative quantities of exudate during 180 min. Each point represents a mean value of six samples; standard deviation indicated by bars.

Table II. Effect of Potassium on the Osmotic Potential and the Concentration of Inorganic Constituents of Phloem Exudate

The concentration of each added component was 1 mM. Values for K, Mg and the osmotic potential are means of 12 samples; other values are means of five samples.

	K Treatment	
	0.4 meq/l	1.0 meq/l
Potassium	47	66**
Sodium	11.5	6.4
Magnesium	3.9	4.1
Calcium	0.65	0.69
Chloride	10.9	11.4
Orthophosphate	5.0	4.2
Osmotic potential, bars	12.5	14.5***

Significant differences ** P<1%
*** P<0.1%

Table III. Effect of Potassium on the Organic Constituents of Exudate

Values for sucrose, glucose 6-P, fructose 6-P and ATP are means of 12 samples; other values are means of six samples.

	K Treatment	
	0.4 meq/l	1.0 meq/l
	mM	
Sucrose	228	238
Raffinose	1.7	2.5*
Glucose 6-P	0.56	0.82***
Fructose 6-P	0.17	0.25**
ATP	0.73	0.72
UTP	0.14	0.15
UDP-glucose	0.45	0.50

Significant differences * P<5%
** P<1%
*** P<0.1%

DISCUSSION

Increasing the K supply to plants substantially increased the quantity of photosynthates exuded in unit time. For most substances analyzed, this increase was mainly due to the increased sap exudation rate, and only in few cases was the concentration of individual organic constituents raised significantly.

Since no microscopic measurements of the phloem were

Table IV. Effect of Potassium on the Content of Amino Acids and Malate in the Phloem Sap

	K Treatment		LSD
	0.4 meq/l	1.0 meq/l	(5%)
	mM		
Gln	134.0	128*	2.1
Glu	12.4	15.5*	1.3
Ser	11.8	10.0*	1.1
Cys	9.1	7.9	1.2
Val	3.7	4.6	0.9
Leu	2.9	5.0*	0.9
Asp	2.4	4.8*	0.6
Asn	3.4	3.1	0.4
Thr	3.3	3.9	0.7
Ala	1.9	2.2	0.3
Pro	2.5	2.5	0.5
Phe	2.4	2.8	0.5
γ-aminobutyric acid	1.0	1.3	0.4
Gly	0.8	0.7	0.3
Total amino acids	191.6	192.3	
Malate	0.83	1.32*	

Significant difference * P<5%

Table V. Effect of Potassium on the Exudation Rate, and on the Concentration of ^{14}C in Exudate and in the ^{14}C-treated Leaf

Values are means of six samples

	K Treatment	
	0.4 meq/l	1.0 meq/l
Volume of exudate, ml	1.61	2.63***
^{14}C in exudate, nCi	9.8	14.7***
^{14}C in treated leaf, nCi	37.6	41.0

Significant difference ***P<0.1%

made, it is difficult to rule out that K also influenced the growth of phloem tissue, which in return could have had an effect on the flow rate of exudate. In such a case, however, a higher flow rate is likely to be associated with a dilution of the exudate. As this was not the case, it seems more probable that the K effect was a direct one.

The results of the ^{14}C experiment (Table V) show that the quantities of labeled C in the treated leaf, 210 min after ^{14}C application, did not differ greatly between treatments, but the quantities of labeled C in the exudation sap differed significantly. The ^{14}C-labeled material in the exudate of the K_1 plant amounted to about 25% of the ^{14}C label present in the treated leaf. The corresponding figure for the K_2 plant is 35%. This indicates that K substantially promoted the export of labeled material out of the treated leaf, and it also shows that K increased the CO_2 assimilation rate.

Table III shows that the concentrations of ATP, UTP, and UDP-glucose in the exudate are very similar for both treatments. Thus the higher exudation rate in the K_2 treatment did not result in a dilution of these coenzymes. Hence, the conclusion is justified that K also promoted the synthesis of ATP, UTP, and UDP-glucose. This statement is supported by the higher concentrations of glucose phosphate, fructose phosphate, and raffinose found in the exudation sap of the K_2 plants. These compounds require ATP for their synthesis.

It is probable that both K effects, the promotion of CO_2 assimilation and the promotion at ATP synthesis, are responsible for the higher flux rate in the phloem. Assimilation provided more photosynthates for phloem-loading and ATP more energy which is required for this process. The higher rate of phloem-loading probably resulted in higher osmotic pressures in the phloem sap. Even in the exudate collected at the stem, a significantly higher osmotic pressure was measured in the K_2 treatment. Thus, the assumption is justified that K increased the osmotic content in the sieve tubes of the source (leaves). This should have promoted water uptake from the surrounding tissues leading to a higher pressure in the sieve tubes of the source. According to Geiger (4), the "push" of the source would thus be enhanced. The finding that K^+ increased the flow rate in the sieve tubes more than it did the concentration of photosynthates in the phloem sap agrees well with experimental results of Ashley and Goodson (1). These authors found a higher flow rate of ^{14}C-labeled material in cotton plants well supplied with K, compared with plants which received a lower K supply. Ashley and Goodson (1) also observed this favorable K effect on translocation in cases where CO_2 assimilation was hardly influenced by K. Similar observations have been reported by Hartt (10) as well as by Mengel and Viro (14). It is, therefore, suggested that it is primarily not the assimilation rate but the higher provision of ATP required for the phloem-loading process which is responsible for the beneficial effect of K on phloem transport. That phloem-loading requires ATP has been recently shown by Sovonick *et al.* (16). The interpretation given here fits in well with the concept of phloem-loading and transport postulated by Hartt (8).

According to Spanner's theory (17), K^+ is directly involved in the transport of photosynthates through the sieve plates. In the experiment discussed here, the higher K treatment increased the K level in the phloem sap by only 50%, whereas the translocation rate was increased by 100%. These data therefore do not support the electroosmotic theory. The results obtained in this experiment are in good agreement with the pressure flow hypothesis as outlined more recently by Zimmermann (19) and Geiger (4).

The concentrations of organic and inorganic solutes found in the exudate are in the same order of magnitude as those reported by Hall and Baker (6) for *R. communis*, with exception of the concentration of total amino acids. In our experiment, the latter was about five times higher than that found by Hall and Baker (6). The significant effect of K on the content of malate in the phloem sap (Table IV) is noteworthy as malate is required to balance K in the phloem sap (2, 11). This effect is probably not very important, at least not in the experiment described here, since the K equivalents found in the phloem sap were about 20 times more than the malate equivalents.

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