A method for determining Michaelis-Menten kinetic parameters of nutrient uptake for plants growing in soil

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Summary - Zusammenfassung

The relationship between nutrient influx (I_n) and solution concentration at the root surface (C_{lo}) has not yet been determined for roots growing in soil because of difficulties in measuring C_{lo}. Corn was grown on two soils with 12 and 21% clay. Each soil had five K levels ranging from low to very high. Potassium influx (I_n) was determined from K uptake between two harvests and root length. C_{lo} was then calculated from the average soil solution concentration and I_n by assuming that diffusion is the main transport mechanism for K to the root. Potassium influx plotted against C_{lo} showed a saturation curve, typical of a Michaelis-Menten kinetic relationship. The Michaelis-Menten uptake parameters, maximum influx (I_{max}) and Michaelis constant (K_m), were obtained by the "Hanes" plot. There was close agreement, without lack of fit, between calculated and observed data. The proposed procedure therefore appears to be suitable for estimating the uptake kinetics of roots growing in soil. Requirements for applications of the method are discussed.

Eine Methode zur Ermittlung der Parameter der Michaelis-Menten-Kinetik an Pflanzen, die im Boden wachsen

Die Beziehung zwischen dem Nährstoffinflux (I_n) und der Konzentration der Bodenlösung an der Wurzeloberfläche (C_{lo}) ist wegen der Schwierigkeit, C_{lo} zu messen, bisher für Wurzeln die im Boden wachsen, nicht bestimmt worden. In dieser Arbeit wird eine Methode vorgestellt, die mit Hilfe von Modellrechnungen C_{lo} bestimmt und anschließend oben genannte Beziehung ermittelt. Mais wurde auf zwei unterschiedlich texturierten Böden (12 und 21% Ton) mit je fünf K-Stufen angezogen. Der K-Influx (I_n) ergab sich aus der K-Aufnahme zwischen zwei Zeitpunkten und der Wurzellänge. C_{lo} wurde dann aus der K-Konzentration der Bodenlösung und I_n errechnet unter der Annahme, daß der K-Transport zur Wurzel vorwiegend durch Diffusion erfolgte. Die Beziehung zwischen dem gemessenen I_n und C_{lo} zeigte eine Sättigungsfunktion, wie sie für die Michaelis-Menten-Kinetik typisch ist. Die Aufnahmeparameter, maximaler Influx (I_{max}) und die Michaelis-Konstante (K_m), wurden daraus mit Hilfe des "Hanes"-Plot ermittelt. Die gute Übereinstimmung im gesamten Kurvenverlauf zwischen den Messdaten und der errechneten Beziehung deuten darauf hin, daß die vorgeschlagene Methode geeignet ist, um die Aufnahmeeigenschaften von Wurzeln, die im Boden wachsen, zu erfassen. Die Bedingungen, die zur Anwendung der Methode notwendig sind, werden in der Arbeit diskutiert.

Introduction

Nutrient influx, i.e. the amount of nutrient taken up per unit of root and time, depends on its concentration at the root surface. This relationship can be described by Michaelis-Menten kinetics (*Epstein* and *Hagen*, 1952).

$$I_n = \frac{I_{max} C_{lo}}{K_m + C_{lo}} \quad [1]$$

where I_n [mol cm^{-1} s^{-1}] is the influx, C_{lo} [mol cm^{-3}]is the nutrient concentration in solution at the root surface, I_{max} [mol cm^{-1} s^{-1}] is the maximum influx at infinitely high concentration, and K_m [mol cm^{-3}] is the concentration where $I_n = 1/2\ I_{max}$.

Plants do not deplete the concentration of a nutrient down to zero, rather net influx stops at a concentration called $C_{l\ min}$. Because of this fact, instead of C_{lo}, the term "C_{lo}-$C_{l\ min}$" is often used in Eq. [1], as first proposed by *Nielsen* (1972).

The uptake parameters I_{max} and K_m are not constant, but vary with plant species, genotype, plant age, soil temperature and nutritional status of the plant (*Jungk* and *Claassen*, 1986). They must therefore be determined for every case where uptake characteristics of roots are of interest. The measurement of these parameters is not possible with roots growing in soil because soil solution concentration at the root surface, C_{lo}, cannot be measured. The aim of this paper is to estimate C_{lo} by means of model calculations and thereby create the possibility of estimating the uptake parameters I_{max} and K_m for plants growing in soil.

Description of the method

The basis for calculating soil solution concentrations at the root surface is the steady state model of *Baldwin* et al. (1973), which was modified by *Barraclough* (1986). The proposed model is valid when the following requirements are met:

(1) Diffusion must be the main transport mechanism of the nutrient from the soil to the root surface. (2) The extension of the depletion zone has to be of similar size as the half distance between roots. (3) Strong competition among roots should not occur.

Barraclough (1986) rearranged the equation of *Baldwin* et al. (1973) for diffusive transport resulting in

$$\Delta C_l = \bar{C}_l - C_{lo} = -\frac{I_n}{4\pi D_l \theta f}\left(1 - \frac{1}{1-\pi r_o^2 L_v}\ln\frac{1}{\pi r_o^2 L_v}\right) \quad [2]$$

where ΔC_l is the concentration difference between the mean soil solution concentration, $\overline{C}_l$, and the concentration at the root surface, C_{lo}. This difference is necessary to maintain the diffusive flux equal to the measured influx, I_n. ΔC_l depends, as already seen, on I_n and on the diffusion coefficient in water, D_l, the volumetric water content, Θ, and the tortuosity factor, f, as well as on the root radius, r_o, and the root length density, L_v, which gives the distance between roots.

The nutrient concentration at the root surface, C_{lo}, is obtained by subtracting the required concentration difference from the mean soil solution concentration. The Michaelis-Menten parameters (K_m, I_{max}) were determined from I_n and its corresponding C_{lo} by using a linear transformation called "Hanes plot" (*Lasch*, 1987). For the "Hanes plot" C_{lo}/I_n is plotted against C_{lo}. The intersection of the regression line of these data with the X-axis gives $-K_m$ and the intersection with the Y-axis gives K_m/I_{max}. The straight line is fitted by linear regression analysis.

Application of the method

To demonstrate the application of the method, data of a pot experiment on K uptake are shown in Table 1. Potassium was chosen because its transport to the root proceeds mainly by diffusion, even at the highest K level. On the other hand, K mobility (effective diffusion coefficients were between 1 and 2 x 10^{-7} cm^2 s^{-1}) was high enough that depletion zones extended to the middle between neighboring roots. Corn was grown on two luvisols derived from loess with five K-levels each. The soils, Bründeln and Bülten, had a clay content of 12 and 21% respectively. Root length, root radius, shoot dry weight and shoot K content were determined 10 and 21 days after sowing. Root length, L, was determined by the method of *Newman* (1966) after washing roots free of soil. An average root radius, r_o, was calculated by Eq. [3] where root fresh weight (RFW) is assumed to be equal to the root volume.

$$r_o = \sqrt{(RFW/L\pi)} \quad [3]$$

Potassium influx was calculated using Eq. [4] (*Williams*, 1946):

$$I_n = (U_2-U_1)(\ln L_2-\ln L_1)/((L_2-L_1)(t_2-t_1)) \quad [4]$$

where U is the K uptake of the shoot, t is time and subscripts 1 and 2 refer to first and second harvest. Soil solution was obtained by the displacement procedure of *Adams* (1974) and its K concentration, C_{li}, determined by flame emission.

The data of Table 1 show that in the low concentration range of C_{lo} an increase in concentration resulted in a marked increase of the influx, I_n. Above a K concentration of 160 µmol l^{-1}, I_n increased only slightly. The data are plotted in Fig. 1, along with the curve obtained from the calculated uptake parameters K_m and I_{max} by using Eq. [1].

There is no lack of fit between the calculated curve and the observed points, i.e. they follow Michaelis Menten kine-

Table 1: Data of calculate C_{lo} from a K-fertilizer experiment with corn. C_{lo} is the concentration at the root surface as obtained by Eq. [2]. The initial concentration of soil solution, C_{li}, is considered as $\bar{C}_l$.

Tabelle 1: Datengrundlage zur Berechnung von C_{lo} aus einem K-Düngungsversuch mit Mais. C_{lo} ist die nach Gleichung [2] errechnete Konzentration an der Wurzeloberfläche. Die Ausgangskonzentration der Bodenlösung, C_{li} steht für $\bar{C}_l$.

soil	fertilization µmol K/100 g	r_o cm x 10^{-2}	I_n mol cm^{-1} s^{-1} x 10^{-14}	ΔC_l	C_{li} µmol l^{-1} K	C_{lo}
Bründeln	-K(1)	1.63	27.2	68	116	48
	0	1.47	39.3	102	266	164
	150	1.55	45.9	117	467	350
	300	1.33	43.5	117	760	643
	450	1.54	50.9	130	1120	990
Bülten	-K(1)	1.36	15.0	40	87	47
	0	1.55	25.1	64	102	38
	150	1.52	38.8	99	260	161
	300	1.42	42.5	112	435	323
	450	1.41	43.0	113	680	567

All soils: $D_l = 1.98 \times 10^{-5}$ $cm^2 s^{-1}$, $\theta = 0.286$ cm^3 cm^{-3}, f = 0.282 and $L_v = 3$ cm cm^{-3}

(1) The soil was depleted of K by preculture of corn.

Für alle Böden: $D_l = 1.98 \times 10^{-5}$ $cm^2 s^{-1}$, $\theta = 0.286$ cm^3 cm^{-3}, f = 0.282 und $L_v = 3$ cm cm^{-3}

(1) Der Boden wurde durch Vorbepflanzung mit Mais an K verarmt.

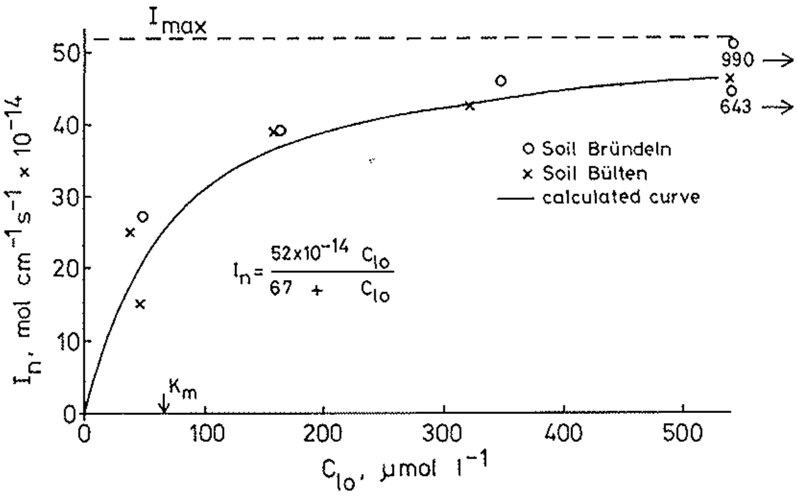

Figure 1: Potassium uptake isotherm of corn roots growing in soil. The K concentration at the root surface, C_{lo}, was calculated with Eq.{2}, and I_{max} [mol cm^{-1} s^{-1}] and K_m [µmol l^{-1}] were obtained by using the Hanes-transformation.

Abbildung 1: Kalium-Aufnahme-Isotherme von Mais im Boden. Die K-Konzentration an der Wurzeloberfläche, C_{lo}, wurde mit Gleichung [2] und I_{max} [mol cm^{-1} s^{-1}] sowie K_m [µmol l^{-1}] mit der Hanes-Transformation ermittelt.

tics as was shown for plants growing in nutrient solution (*Epstein* and *Hagen*, 1952). The uptake parameters I_{max} and K_m are of the same order of magnitude as data from nutrient solution experiments (*Jungk* and *Claassen*, 1986). The proposed method therefore appears to be suitable to estimate the uptake characteristics of roots growing in soil.

$C_{l\ min}$, the concentration where net influx equals zero, was not taken into account because it cannot be measured in experiments with soil. However, influence of $C_{l\ min}$ would be negligible, when K_m is as high as observed and $C_{l\ min}$ as low as 1 - 2 µmol l^{-1} as was reported by *Barber* (1984) for solution culture experiments. Nevertheless, C_{lo} should be corrected for $C_{l\ min}$ if data are available.

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