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Determination of kinetic parameters for potassium uptake by wheat at different growth stages

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Abstract

Root influx parameters (V_{max} and K_m) of wheat (*Triticum aestivum* var. HD-2285) were determined at different stages of crop growth, viz. *CRIS*-Crown Root Initiation Stage, *MTS*-Maximum Tillering Stage, *FLS*-Flag Leaf Stage and *DFS*-Dough Formation Stage. Wheat was grown in sand medium with Hoagland nutrient solution (all nutrients except potassium). Plants were taken out from sand culture at 22, 41, 69, and 87 days after germination (*DAG*) and placed in a specially designed assembly of flowing solution in laboratory and greenhouse. The nutrient solution allowed to flow into the culture vessel in a regulated manner by means of a separating funnel. Fresh Hoagland solutions having five different concentration of K^+ (0.051, 0.154, 0.256, 0.333, and 0.410 m*M*) were added drop-wise on the funnels at the rate of about 35 µL s^{-1} and continuously supplied in five different assemblies. Root influx parameters (V_{max} and K_m) were determined employing a simple solution culture technique, by measuring the depletion of potassium (input-output concentration) by wheat roots in five nutrient media differing in potassium concentration only. V_{max} and K_m were calculated with the help of coefficients obtained by fitting the solution K^+ concentration and K^+ uptake rate data on the Michaelis-Menten equation. The V_{max} and K_m values were decreased with the age of wheat crop. The V_{max} was 48.7 nmol m^{-2} s^{-1} at 22 DAG and decreased to 19.4 nmol m^{-2} s^{-1} at 41 DAG, 5.90 nmol m^{-2} s^{-1} at 69 DAG and finally decreased to 4.21 nmo1m^{-2} s^{-1} at 87 days of crop growth; corresponding K_m values were 0.299, 0.254, 0.176 and 0.146 m*M*, respectively.

Keywords: K_m, and V_{max}; Michaelis-Menten constant; Root influx parameter; Wheat

Introduction

Since the middle of 19th century, the experimental culture of plants in soil-less media has become a widespread approach to the study of physiological phenomena, especially those centered on root activity and function. In the solution culture method, plant roots are completely immersed in aqueous solution in which mineral nutrients are dissolved. Solution culture methods are widely used for studying root physiology, and are especially critical to the study of plant mineral nutrition. They provide an obvious advantage of a well-defined,

homogeneous, and fully controllable medium in terms of its elemental composition. Flowing solution culture represents one extreme case in nutrient buffering where solution concentration is very low and very stable (Parker and Norvel, 1999).

Claassen and Barber (1977) reported that, at K^+ levels usually found in soil solution, K^+ influx characteristics of plant root could be described by Michaelis-Menten kinetics. When only part of the roots are supplied with K^+, plant uptake of K^+ may be reduced, causing a reduction in K^+ concentration in the plant and may also reduce plant growth. Nitrogen influx was greater on the K^+ present than on the K^+ absent side, but presence of K^+ did not influence P uptake and Mg uptake was greater where K^+ was absent.

Datta and Sastry (1988) worked on a simple solution culture technique for raising wheat plants in three nutrient media differing in respect of potassium concentrations only. They reported that potassium content and dry weight of plants increased significantly with rise in K^+ concentration in the nutrient media while the percentage of N (in plant) did not vary with solution K^+ level. Results of flowing solution culture experiment compared favorably with those obtained with wheat grown in an alluvial soil.

Seward et al. (1990) attempted modeling K^+ uptake by wheat. Spring wheat was grown in the field under deficient and sufficient levels of soil K^+ and with high and low supply of fertilizer nitrogen. Their model, predicted K^+ uptake reasonably well where uptake was expected to be determined by supply, that is the low level of K^+ treatments, but where plant demand was expected to be controlling uptake, that is in the high level of K^+ treatments, the model did not predict K^+ uptake very well. They reported that over-prediction would occur if measured root length was not all active in uptake, or if older roots were less efficient, or if maximum influx (I_{max}) were too large.

Potassium uptake in the roots of intact 6-day-old wheat seedlings grown on low-salt solutions was investigated by Kosourov et al. (1999). When the potassium concentration exceeded 34 μ*M,* a gradual acceleration of K^+ uptake was recorded at the first stage (20-75 min). This stage became longer at higher initial concentrations of potassium in the nutrient solution. The period of initial acceleration of potassium uptake was almost indistinguishable at 16 μM K^+. At low K^+ concentrations (below 30-34 μM), the kinetic curves were adequately approximated by the equation for first-order reactions. At higher concentrations (30-190 μM), the isotherm for potassium uptake was more precisely approximated by a linear equation. With some limitations, the isotherm might be approximated by a hyperbola basing on Michaelis-Menten equation with a high K_m of about 650 μM. The high value of K_m and initial acceleration of potassium uptake suggest an induction or activation of potassium transport systems at the first stage by ambient potassium, when its concentration exceeds 30-34 μM.

Trehan and Classen (2001) worked out on potassium uptake kinetics of potato, wheat and sugar beet plants as affected by the K nutritional status. In their study K^+ uptake kinetic parameters i.e. maximum influx per unit root length (I_{max}), the Michaelis-Menten constant (K_m) and the minimum concentration (C_{min}) were measured for potato and wheat plants (28 days old) and for sugar beet plants (23 days old), in order to elucidate the basis of these differences. These plants were grown at constant concentration of 1.5, 15 or 200 μM K in flowing solution prior to start of depletion. I_{max} was higher in plants grown previously at low K^+ concentration than in plants grown at higher K concentration for all the plant species. I_{max} was negatively correlated to extent of K^+ sufficiency (shoot K^+ concentration

expressed as percent of minimum required for maximum shoot growth rate of each species) irrespective of plant species. In contrast to the hypothesis, K^+ uptake kinetics parameters of potato were similar to those of wheat and sugar beet at the age of their measurement. This contradiction seems to be based on the fact that potato became adapted to a low K^+ supply by improving its uptake kinetic parameters, mainly the I_{max} value with time. In this way after 4 weeks of adaptation, potato had reached a similar uptake capability per unit of root as the other species.

Roshani et al. (2009) used a new mathematical model to simulate potassium uptake by wheat crop using three different soils (Alfisol, Inceptisol and Vertisol). The model determined 16 different parameters such as: initial available K; buffer power of nutrient on the solid phase for nutrient in solution; fixation threshold level; release threshold level; fixation rate constant; release rate constant; rate of root's water uptake; maximum root K uptake rate (V_{max}); soil solution K concentration at which root K uptake rate was half that of maximum K uptake rate (K_m); and minimum soil solution K concentration at which net influx is equal to efflux rate (C_{min}). Sensitivity analysis indicated that K uptake increased rapidly with increasing mean root radius and V_{max} suggesting the importance of root surface area. They shown that, the potassium uptake by wheat was least affected by C_{min}.

In this experiment, kinetic parameters of potassium uptake by wheat at different growth stages were studied and root influx parameters (V_{max}, and K_m) were determined at different stages, viz. *CRIS*-Crown Root Initiation Stage, *MTS*-Maximum Tillering Stage, *FLS*-Flag Leaf Stage and *DFS*-Dough Formation Stage.

Materials and Methods

For determining the net maximum influx rate per unit volume of root (V_{max}) and Michaelis-Menten constant (K_m), a solution culture experiment was carried out. Kinetic parameters of potassium uptake by 22, 41, 69, and 87-day-old wheat seedlings were determined. Initially wheat seedlings were grown in a sand culture with complete Hoagland solution (all nutrients except potassium). Root influx parameters (V_{max} and K_m) were determined employing a simple solution culture technique, by measuring the depletion of potassium (input-output concentration) by wheat roots in five nutrient media differing in potassium concentration only. The kinetics of potassium uptake depended on the initial K^+ concentration in the solution. Potassium uptake by wheat is calculated with the help of solution K^+ concentration, volume of sample collected, root surface area and time intervals at which samples were collected at 22, 41, 69, and 87 days after germination. Five different concentrations of K^+, i.e. 0.051, 0.154, 0.256, 0.333, and 0.410 m*M* were maintained in continuous flowing Hoagland's K free nutrients solution in five different assemblies.

Wheat (*Triticum aestivum* var. HD-2285) was grown in sand medium with Hoagland nutrient solution (all nutrients except potassium, Table 1). Nutrient solution was drained out every day before fresh addition to maintain uniform concentration around roots and to avoid production of any allelopathic compounds in solution, which would inhibit nutrient absorption. Plants were taken out from sand culture at 22, 41, 69, and 87 days after germination (*DAG*) and placed in a specially designed assembly of flowing solution (Figure 1) in laboratory and greenhouse. The nutrient solution was allowed to flow into the culture vessel in a regulated manner by means of a separating funnel. The set-up was in the form of

a U-tube, one arm of which in the shape of a large glass funnel where plants were placed with proper support. Fresh Hoagland solution was added drop by drop and used up liquid was collected intermittently from the other end of the tube.

Table 1. Composition of used Hoagland solution.

Compounds	Concentration of stock solution	Volume of stock solution per litre of final solution	Element	Final concentration of element (ppm)
Macronutrients				
KNO_3	1*M*	6.0 mL	N	224
$Ca(NO_3)_2.4H_2O$	1*M*	4.0 mL	K	235
$NH_4H_2PO_4$	1*M*	2.0 mL	Ca	160
$MgSO_4.7H_2O$	1*M*	1.0 mL	P	62
			S	32
			Mg	24
Micronutrients[1]				
KCl	50 m*M*		Cl	1.77
H_3BO_3	25 m*M*		B	0.27
$MnSO_4.H_2O$	2.0 m*M*	1.0 mL	Mn	0.11
$ZnSO_4.7H_2O$	2.0 m*M*		Zn	0.131
$CuSO_4.5H_2O$	0.5 m*M*		Cu	0.032
H_2MoO_4	0.5 m*M*		Mo	0.05
Fe-EDTA[2]	20 m*M*	1.0 mL	Fe	1.12

[1]A combined stock solution was prepared containing all micronutrients except iron.
[2]Ferrous dihydrogen ethylendiamine tetraacetic acid.

(a) (b) (c)

Figure 1. (a) Experimental setup in laboratory with artificial lighting, (b) measurement of light intensity in greenhouse, and (c) the experimental setup was shifted from laboratory to greenhouse.

During daytime, no special arrangement for bubbling of air or stirring the solution was made because the solution was continuously flowing which prevented the development of concentration gradient around the roots as well as depletion of oxygen in the nutrient media. Furthermore, flow of nutrient solution removed some allelopathic compounds, if

any, secreted by the roots. But in night time, an aquarium pump was used to make bubbling around the roots to avoid the above stated undesirable developments. Fresh Hoagland solutions having five different concentration of K^+ (51, 154, 256, 333, and 410 μ*M*) were added drop-wise on the funnels at the rate of about 35 μL s^{-1} and continuously supplied in five different assemblies. In laboratory, an artificial light was provided to the plants for maintaining their normal photosynthesis rate but because of insufficient amount of light intensity, which has been measured by a digital Lux-meter, the set-up was transferred to the greenhouse.

At different growth stages, after running the experiment for 24 h, outlet solution was collected and the rate of K^+ absorption was determined from the difference in concentration at the inlet and outlet. At each stage, the surface area of the absorbing roots was also calculated from its fresh root weight (Nye and Tinker, 1977). The values of V_{max} and K_m at each growth stage were calculated from the slope and intercepts of the linear equation fitted by plotting C/V against C, where V is the rate of K^+ absorption when its concentration in solution is C (Figure 2). V_{max} and K_m, values obtained at different stages were fitted to exponential equation with coefficients of A and B as shown below:

$V_{max} = A_V \exp(-B_V t)$

$K_m = A_K \exp(-B_K t)$

where, A_V and A_K are the values of V_{max} and K_m at the start of simulation and B_V and B_K are constants analogous to decay constant of V_{max} and K_m, respectively, with time, *t*, of growth.

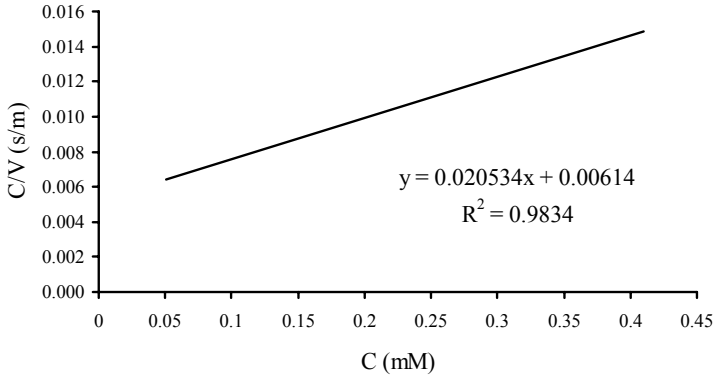

Figure 2. The relationship between C/V and C at crown root initiation stage.

Results

Solution K^+ concentrations and potassium uptake rates at different growth stages of crop were fitted in Michaelis-Menten equation. Then the maximum influx rate per unit volume of the roots (V_{max}) and the Michaelis-Menten constant (K_m) were calculated with the help of fitted coefficients obtained by fitting the solution K^+ concentration and potassium uptake rate data on the Michaelis-Menten equation. At 22, and 41 days after germination in wheat,

corresponding to crown root initiation stage and maximum tillering stage, the maximum potassium uptake per unit surface area of the roots (28.3 and 13.4 nmol m^{-2} s^{-1}) were noticed at 0.41 m*M* solution K^+ concentration. At concentrations lower than 0.41 m*M* of K^+, its uptake rate increased gradually but at 69 and 87 days after germination (i.e., flag leaf stage and dough formation stage), the potassium uptake rate increased very slowly (Figure 3).

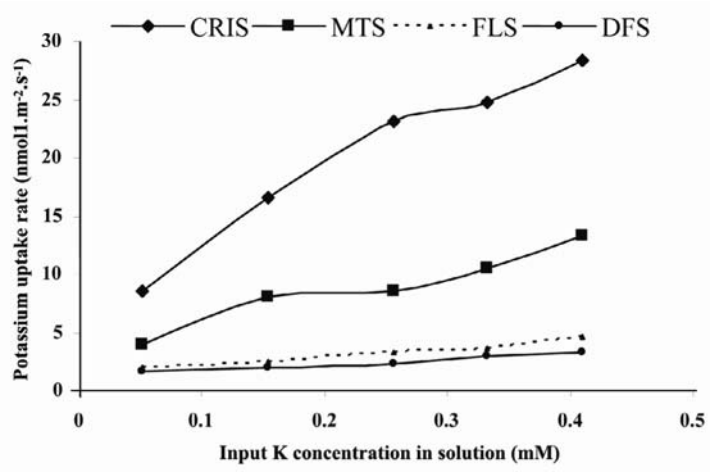

Figure 3. Rate of K^+ uptake (nmol.m^{-2}.s^{-1}) at different solution K^+ concentration (m*M*) during different growth stages of wheat.

The V_{max} and K_m values of wheat crop are tabulated in Table 2. It is evident from the table that the V_{max} and K_m values were decreased with the age of wheat crop. The V_{max} was 48.7 nmol m^{-2} s^{-1} at crown root initiation stage (22 *DAG*) and decreased to 19.4 nmol m^{-2} s^{-1} at maximum tillering stage (41 *DAG*), 5.90 nmol m^{-2} s^{-1} at flag leaf stage (69 *DAG*) and finally decreased to 4.21 nmo1m^{-2} s^{-1} at dough formation stage (87 *DAG*); corresponding K_m values were 0.299, 0.254, 0.176 and 0.146 m*M*, respectively (Table 2 and Figure 4).

Table 2. Values of V_{max} and K_m for potassium uptake equation at different growth stages of wheat.

Growth stage of wheat[1]	Solution K^+ concentration (mM)					V_{max} (nmol m^{-2} s^{-1})	K_m (m*M*)
	0.051	0.154	0.256	0.333	0.410		
	Uptake rate (nmol m^{-2} s^{-1})						
CRIS	8.49	16.5	23.1	24.7	28.3	48.7	0.299
MTS	3.89	8.04	8.58	10.5	13.4	19.4	0.254
FLS	1.93	2.42	3.20	3.59	4.58	5.90	0.176
DFS	1.57	1.97	2.32	2.97	3.34	4.21	0.146

[1]Crown Root Initiation Stage (*CRIS*), Maximum Tillering Stage (*MTS*), Flag Leaf Stage (*FLS*), and Dough Formation Stage (*DFS*) were 22, 41, 69, and 87 days after germination.

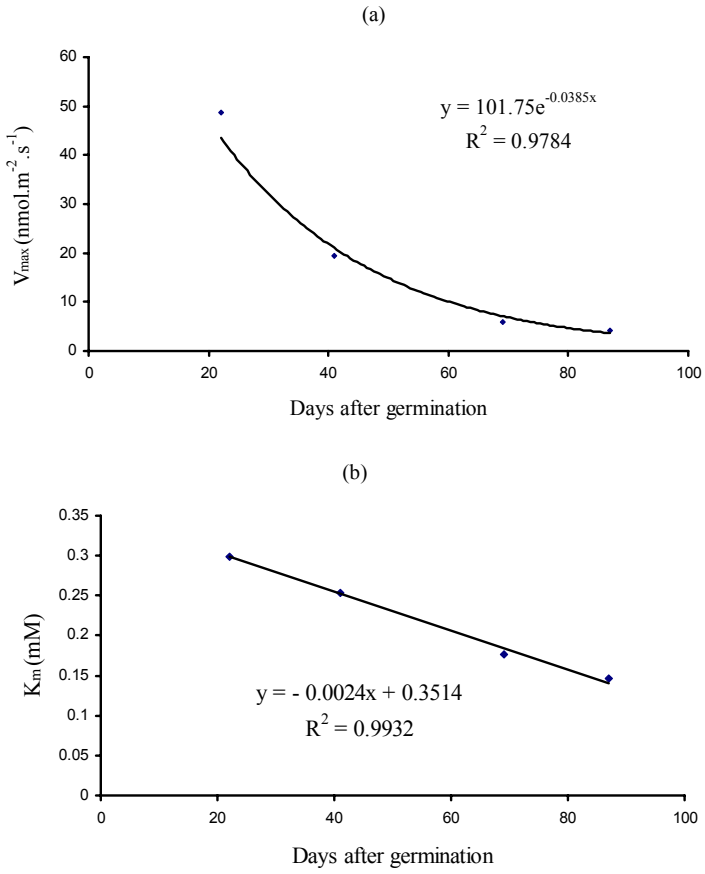

Figure 4. Relationship between wheat age and changes in V_{max} (a) and K_m (b).

Discussion

The potassium uptake efficiency at different stages of plant growth can be characterized by the K^+ content when plants are growing under the same conditions and where potassium is limiting growth. As it is evident from the Figure 5, during the first two-three hours after transfer of seedlings from sand culture to the solution culture, and in the time of new sample collection (early morning) potassium efflux took place (may be because of transfer shock). However, when the potassium concentration in the nutrient solution exceeded 0.050 m*M*, a gradual acceleration of K^+ uptake was recorded.

Similar to initial growth stage (22 *DAG*), potassium uptake rates decreased at higher K^+ concentration (≥ 0.256 m*M*) in nutrient solution. Overall potassium uptake was reduced at 69 *DAG* from that of 41 and 22 *DAG* of the crop. After 69 *DAG*, maximum and minimum potassium uptake rates were found (3.34 and 1.57 nmol m^{-2} s^{-1}) at 0.410 and 0.051 m*M* solution K^+ concentration, respectively. Further, at the late stage of the crop growth, the

matured K^+ uptake rate per unit surface area of root decreased drastically at all solution K^+ concentrations. As it is evident from the Figure 4 (graphs A and B), there is a significant negative correlation between wheat crop age and the values of root influx parameters (V_{max} and K_m). The decrease in V_{max} and K_m values with increasing of wheat crop age follows the different equations, which are exponential, and linear equation, respectively.

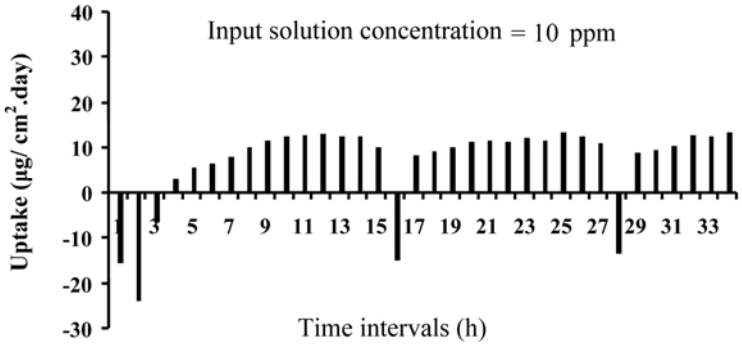

Figure 5. Relationship between transferring time of the seedling from sand culture to the funnels and potassium uptake by wheat crop.

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