棉花钾吸收动力学的初步研究和应用

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摘要：研究了影响棉花钾吸收动力学参数的因素，初步确定了钾吸收动力学方法在棉花上应用的条件，比较了转基因抗虫棉新棉99B和常规棉中棉所35苗期的钾吸收特性。结果表明，棉花钾吸收动力学参数受到苗龄、耗竭液中起始钾浓度及培养液中钾浓度的显著影响，在苗龄较小(3～4叶苗与4～5叶苗相比)、耗竭液中起始钾浓度较高(0.35与0.2 mmol·L^{-1}相比)、培养液中钾浓度较高(2.0与0.5 mmol·L^{-1}相比)的情况下，K_m、C_{min}均比较高，而I_{max}在前两种条件下较高、在后一种条件下较低。在5～6叶期，新棉99B的I_{max}显著降低于中棉所35，而K_m、C_{min}显著高于后者(培养液中K^+为0.5 mmol·L^{-1})，耗竭液中起始K^+为0.2 mmol·L^{-1})，说明新棉99B吸收有限钾的能力低于中棉所35。

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Preliminary Study of K^+ Uptake Kinetics of Cotton (*Gossypium hirsutum* L.) and its Application

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Abstract: It has been a main problem in cotton production that transgenic insect-resistant cotton is prone to premature senescence induced by K^+ deficiency, which makes it necessary to study K^+ uptake characteristics and, on the basis of it, to screen cotton cultivars with high K^+ uptake efficiency. This experiment studied the factors influencing K^+ uptake kinetic parameters of cotton, preliminarily identified the conditions of K^+ uptake kinetic applied to cotton, and compared the K^+ uptake characteristics of transgenic insect-resistant cotton cultivar, DP99B, and conventional cotton cultivar, CCRI 35, at seedling stage. The results showed that K^+ uptake kinetic parameters were significantly affected by seedling stage, initial K^+ concentration in depleting solution and K^+ concentration in culturing solution. Higher K_m and C_{min} resulted from younger seedling (3～4 vs. 4～5 leaf stage), higher initial K^+ concentration in culturing solution (0.35 vs. 0.2 mmol·L^{-1}) and higher K^+ concentration in culturing solution (2.0 vs. 0.5mmol·L^{-1}), but higher I_{max} was produced under the two former conditions, and lower I_{max} came into being under the last situation. Compared with CCRI 35, DP99B had significantly lower I_{max} and higher K_m and C_{min} (0.5 mmol·L^{-1} K^+ in culturing solution and 0.2 mmol·L^{-1} initial K^+ in depleting solution) at 5～6 leaf stage, which indicated that the ability of DP99B to uptake limited K^+ was weaker than that of CCRI 35.

Key words: cotton; K^+; uptake kinetics

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1 Introduction

Since enzymatic reaction kinetics, by using *in vitro* root and radio-labeled rubidium [1] and/or *in vivo* root and non-radio-labeled potassium, named 'ion depletion method' [2], was used to study potassium uptake of plant roots, a lot of studies have been carried out, including differences of intra-species and inter-species in K^+ uptake parameters I_{max} (K^+ maximum uptake rate), K_m (K^+ concentration in solution where K uptake rate was half of I_{max}) and C_{min} (where K influx equaled efflux)[3], effects of seedling age[4], internal K^+ content of seedling[5,6] and external environments[7] etc. on I_{max}, K_m and Cmin. I_{max} represented maximum absorbing rate, which mattered when K^+ supply was abundant. K_m represented competitive capacity of plant for limited K^+ source, which meant that the lower K_m was, the stronger the competitiveness for K^+ was. C_{min} was the minimum K^+ concentration below which K^+ couldn't be absorbed by root. When K^+ available in medium was limited, K_m and C_{min} greatly mattered. It has showed that I_{max}, K_m and C_{min} varied in a wide range among varieties and species, and these K^+ uptake kinetic parameters could be effective tools to select crop varieties with high potassium efficiency for breeder and growers [8].

Cotton had less root density than other crops [9], which determined its relatively low K^+ uptake efficiency. Recently, premature senescence induced by potassium deficiency frequently happened to cotton, especially to transgenic insect-resistant cotton. Therefore, it was necessary to study the mechanisms of K^+ uptake of cotton. But up to now, few studies of K^+ uptake kinetics of cotton were found.

The objectives of this experiment were to investigate the effects of seedling age, initial K^+ concentration in the depleting solution and K^+ concentration in culturing solution, on the K^+ uptake kinetic parameters, based on that, to analyze the differences of K^+ uptake kinetic characteristics between conventional variety CCRI 35 and transgenic insect-resistant cotton DP99B and to identify the conditions of K^+ uptake kinetic applied to cotton.

2 Materials and methods

2.1 Solution culture

The experiments were conducted in a growth chamber at 30℃ day/25 ℃ night, 10 h light/14 h dark condition using transgenic insect-resistant cotton DP99B and conventional variety CCRI 35. Photo-radiation at the canopy was 450 $\mu mol \cdot m^{-2} \cdot s^{-1}$. The seeds were surface sterilized by 9% H_2O_2, germinated and emerged in 1/4 modified Hoagland's solution described below without K^+. Five-day-old seedlings were carefully transferred into $35 \times 27 \times 12\ cm^3$ pots rounded with aluminum foil. The pots were loaded with a modified Hoagland's solution, which contained 2.5 $mmol \cdot L^{-1}$ $Ca(NO_3)_2$, 1 $mmol \cdot L^{-1}$ $MgSO_4$, 0.5 $mmol \cdot L^{-1}$ $(NH_4)H_2PO_4$, 2×10^{-4} $mmol \cdot L^{-1}$ $CuSO_4$, 1×10^{-3} $mmol \cdot L^{-1}$ $ZnSO_4$, 0.1 $mmol \cdot L^{-1}$ EDTA Fe Na, 2×10^{-2} $mmol \cdot L^{-1}$ H_3BO_3, 5×10^{-6} $mmol \cdot L^{-1}$ $(NH_4)_6Mo_7O_{24}$, 1×10^{-3} $mmol \cdot L^{-1}$ $MnSO_4$ and varied KCl for different K^+ treatments. All solutions were changed twice a week and de-ionized water was added daily to replace the water lost by evapo-transpiration. The pH was maintained close to 6.5 by adding H_2SO_4. Air was bubbled into the nutrient solution by air pump to provide O_2 and mix solution homogeneously.

2.2 Determination of K^+ uptake kinetic parameters and K^+ content in root

K^+ uptake kinetic parameters were determined by using ion-depletion method[2]. Three uniform seedlings were selected as a unit, and put into 500 ml beakers loaded with 300 ml above described nutrient solution without K^+, which was hereby defined as K^+ starvation of seedlings. After 48 hours, the seedlings were taken out from the solution. Their roots were washed with 0.5 mmol · L^{-1} $CaSO_4$ three times. Then the seedlings were again put into the beakers with 300 ml uptake solution (hereon called depleting solution) comprised of different initial KCl content and 0.5 mmol · L^{-1} $CaSO_4$, where Ca^{2+} can guarantee integrity of membranes and minimize ions efflux[10]. At 10～30 min intervals, 1.5 ml solution was taken out. At intervals of 30 minutes, 5 ml ionized water was added against evapo-transpiration. After some duration, the procedure was stopped. Seedlings were taken out and weighed for fresh root weight. Root was oven-dried at 75℃, grounded, and K^+ was extracted from root with 1 mmol · L^{-1} ammonium acetate (pH = 7.0). Extracted K^+ of root and K^+ in taken-out solution were determined by atomic absorption spectroscopy (Varian SpectrAA). During depletion, K^+ concentration in solution decreased as a binomial function of time, so I_{max}, K_m and C_{min} were calculated according to the following formulae:

$$\text{Conc.} = A * t^2 + B * t + C$$

$$I = -d\,\text{Conc.}/d\,t = -2 * A * t - B$$

$$I_{max} = |B| * V/FRW$$

$$K_m = B^2/16A - B^2/4A + C$$

$$C_{min} = B^2/4A - B^2/2A + C$$

Note: Conc.: K^+ concentration in the depleting solution; t: time; A, B: coefficients; C: constant; I: K^+ influx rate; I_{max}: maximum influx rate; K_m, C_{min}: K^+ concentration in the depleting solution where I was a half of I_{max} and tended to zero, respectively; V: the volume of the depleting solution; FRW: fresh root weight.

2.3 Experiment designs and statistical analysis

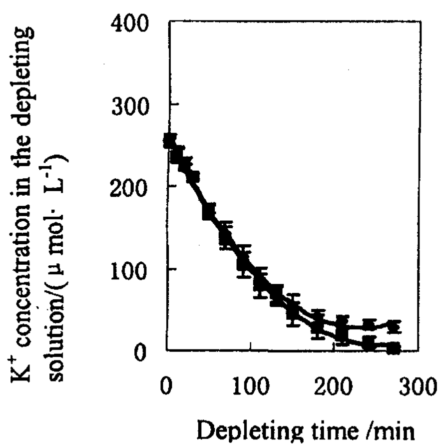
In this experiment, effects of seedling age (sub-experiment 1), K^+ concentration in culturing solution (sub-experiment 2) and initial K^+ concentration in depleting solution (sub-experiment 3) on parameters of K^+ uptake kinetics were investigated. Further, K^+ uptake kinetic parameters of CCRI 35 and DP99B were compared (sub-experiment 4). At seedling age, K^+ concentration in culturing solution and initial K^+ concentration in depleting solution of each sub-experiment were detailed in table 1.

A completely randomized design with three replications was used for each of four experiments. All data were subject to ANOVA test and means were compared using the Fisher's protected LSD ($P < 0.05$).

3 Results

3.1 Factors influencing K^+ uptake kinetic parameters

3.1.1 *Seedling age*. When seedlings growing from 3-4 leaf to 4-5 leaf, fresh root weight increased by 70.8%, K^+ concentration in root decreased by 19.1%, and I_{max}, K_m and C_{min} decreased by 41.4%, 17.7% and 72.7%, respectively. The decrease of K_m and C_{min} indicated the competitive uptake on limited K^+, which should result mainly from the decrease of K^+ concentration in root (Table 2 and Figure 1).



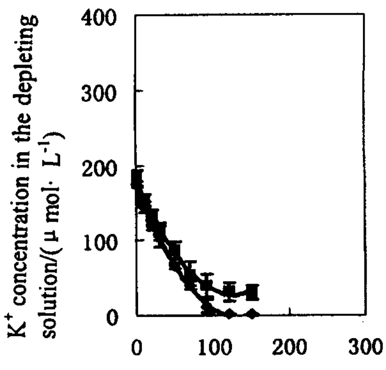
Diamond symbol (◆): seedlings at 3-4 leaf stage; Foursquare symbol (■): The seedlings at 4-5 leaf stage. (Error bars signified ± S. E of means. The same for the following figures)

Fig. 1 K^+ uptake kinetics curve of seedlings at different growth stages.

3.1.2 *K^+ concentration in the culturing solution*. Seedlings with 2 mmol · L^{-1} K^+ had significantly 68% higher fresh root weight and 191.8% higher K^+ concentration in root, on the other hand, had 40% lower I_{max} and 55.9% higher K_m and 896% higher C_{min}, compared with seedlings cultured with 0.5 mmol · L^{-1} K^+ in solution. This indicated that the K^+ uptake ability reduced as a result of K^+ concentration increment in root (Table 2 and Figure 2), which was accordant with the above result.

Table 1 Seedling age, K^+ concentration in culturing solution and initial K^+ concentration in depleting solution of each sub-experiment

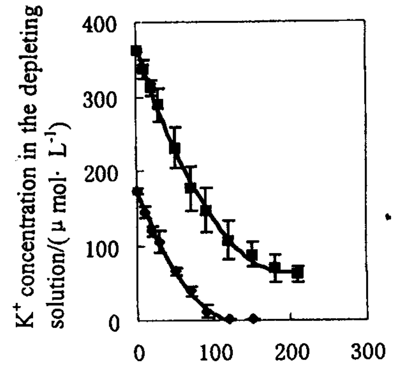
	seedling age true leaf	K^+ in culturing solution /(mmol · L^{-1})	initial K^+ in depleting solution /(mmol · L^{-1})
sub-experiment 1	3～4 and 4～5	0.5	0.25
sub-experiment 2	5～6	0.5 and 2.0	0.20
sub-experiment 3	5～6	0.5	0.20 and 0.35
sub-experiment 4	5～6	0.5	0.20



Diamond symbol (◆): seedlings cultured with 0.5 mmol · L^{-1} K^+; Foursquare symbol(■): The seedling cultured with 2 mmol · L^{-1} K^+

Fig. 2 K^+ uptake kinetics curve of seedlings cultured with different K^+ concentration in culturing solution

3.1.3 *Initial K^+ concentrations in depleting solution.* With seedlings cultured in same conditions, higher initial K^+ concentration (0.35 vs. 0.2 mmol · L^{-1}) during depletion made K^+ concentration in root, I_{max}, K_m and C_{min} elevated significantly by 84.3%, 20.0%, 24.4% and 255.6%, respectively. This suggested that higher exterior K^+ raised the initial rate and accumulation of K^+ influx into root during depletion, which further inhibited the K^+ uptake, and produced higher K_m and C_{min} (Table 2 and Figure 3).



Diamond symbol(◆): 0.2mmol · L^{-1} K^+; Foursquare symbol(■): 0.35mmol · L^{-1} K^+

Fig. 3 K^+ uptake kinetics curve of seedlings with different initial K^+ concentration in depleting solution

Table 2 Effects of seedling stage, K^+ concentration in culturing solution and initial K^+ concentration in depleting solution on K^+ uptake kinetic characteristics; K^+ concentration in dry root and fresh root weight

factors of affecting K^+ uptake kinetics	K^+ uptake kinetics parameters			K^+ content in dry root	Fresh root weight
	I_{max} /μmol · (g · min)$^{-1}$(FRW)	K_m (μmol · L^{-1})	C_{min} /(μmol · L^{-1})	/%	/g
seedling age					
3-4 leaf	0.29A	85.25A	26.85A	3.03A	0.79B
4-5 leaf	0.17B	70.17B	7.32B	2.45B	1.35A
K^+ concentration in culturing solution					
0.5 mmol · L^{-1}	0.10A	40.32B	2.50B	1.47B	2.49B
2.0 mmol · L^{-1}	0.06B	62.86A	24.90A	4.29A	4.20A
initial K^+ concentration in depleting solution					
0.20 mmol · L^{-1}	0.10B	40.32B	2.50B	1.47B	2.49A
0.35 mmol · L^{-1}	0.12A	138.72A	66.41A	2.71A	2.56A

Note: Values in each column followed the upper-case letters for comparisons within factors affecting K^+ uptake kinetics at $P<0.05$ by LSD test.

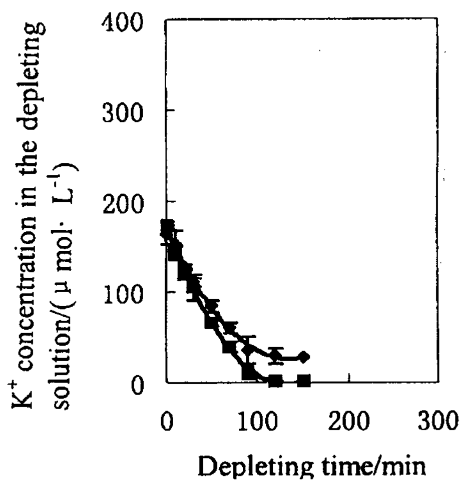
Table 3 Comparisons of K^+ uptake kinetic characteristics, fresh root weight and K^+ concentration in root between two cotton cultivars

varieties	K^+ uptake kinetics parameters			K^+ content in dry root	fresh root weight
	I_{max}(FW) /μmol·(g·min^{-1})	K_m /(μmol·L^{-1})	C_{min} /(μmol·L^{-1})	/%	/g
CCRI 35	0.10A	40.32B	2.50B	1.47B	2.49A
DP99B	0.07B	61.72A	27.50A	1.96A	2.92A

Note: Values in each column followed the upper-case letters for comparison between two cultivars at $P<0.05$by LSD test.

3.2 Comparisons of K^+ uptake kinetic characteristics between CCRI 35 and DP99B

Compared with transgenic insect-resistant cotton DP99B, conventional variety CCRI 35 had similar fresh root weight, 25% lower K^+ concentration in root, but significantly 42.8% higher I_{max} and 34.7% lower K_m and 90.9% lower C_{min}. As mentioned above, lower K^+ concentration in root maybe contributed to the higher I_{max} and lower K_m and lower C_{min}(Table 3 and Figure 4). Furthermore, this result implied that conventional variety CCRI 35 could use limited K^+ more efficiently than transgenic insect-resistant cotton DP99B.



Diamond symbol(◆): DP99B; Foursquare symbol(■): CCRI35

Fig. 4: K^+ uptake kinetics curve of cotton cultivars, DP99B and CCRI 35

4 Discussions

4.1 Conditions of K^+ uptake kinetics applied to cotton

In order to obtain desirable kinetic curve, it was necessary to get the absorption rate varied gradually from rapidly to slowly. Factors influencing K^+ uptake kinetic should contain seedling age, seedling number as a unit, volume and initial K^+ concentration of depleting solution, K^+ concentration in culturing solution (this resulted in K^+ concentration variation in root) and certain starvation time (this guaranteed I_{max} available). The results of this experiment indicated that relatively appropriate K^+ uptake kinetic curves of cotton was obtained with 3-6 leaf seedling under conditions as follows: 450 μmol·m^{-2}·s^{-1} of photo-radiation, 0.5 or 2 mmol·L^{-1} K^+ of culturing solution, 48 hours of seedlings starvation, 3 plants as a unit, 300 ml K^+ depleting solution with 0.2, 0.25 or 0.35 mmol·L^{-1} of initial K^+.

K^+ concentration in root decreased with seedling age, which might be a dilution of rapid root growth, a growth from 0.79 g·plant^{-1} at 3~4 leaf stage to 1.35 g·plant^{-1} at 4~5 leaf stage. But the difference of K^+ concentration in root between two seedling stages couldn't completely account for the bigger differences in I_{max}, K_m and C_{min}. They all significantly decreased with seedling growing, which didn't completely accord with the results from Peng [4], who suggested that there was a small reduction in I_{max} and a slow ascension in K_m with plant age increasing.

There was a positive correlation between increment of K^+ influx and reduction of K^+ concentration in roots [5,6]. To some extent, internal K^+ of root increased, K_m increased [6]. In this experiment, while K^+ concentration in culturing solution increased from 0.5 mmol·L^{-1} to 2 mmol·L^{-1}, the fresh root weight and K^+ concentration in root of seedlings got significantly enhanced, which led to significant increment of K_m and C_{min}, and significant decrease of I_{max}. It was manifested that K^+ uptake efficiency was regulated strongly by feed back of internal K^+ of root.

With initial K^+ enhanced from 0.2 mmol • L^{-1} to 0.35 mmol • L^{-1} during depletion, I_{max} was improved significantly, while K_m and C_{min} were increased significantly and strongly. Those changes may attribute partly to significant disparity of K^+ concentration among seedlings in different initial K^+ concentrations during depletion, which provided another evidence for the feed back regulation of internal K^+ of root on K^+ uptake.

Considering the K^+ concentration in natural soil solution and obvious effects of higher initial K^+ in depleting solution on K_m and C_{min}, it may be appropriate to select cotton varieties with high potassium efficiency under the following conditions: 5～6 leaf seedlings, 0.5mmol • L^{-1} K^+ in culturing solution and 0.2 mmol • L^{-1} initial K^+ in depleting solution.

4.2 Comparison of K^+ uptake kinetic characteristics between CCRI 35 and DP99B

Taking seedlings grown in Hoagland's solution with 0.5 mmol • L^{-1} K^+ for comparisons, great gaps existed between CCRI 35 and DP99B in K^+ uptake kinetic characteristics. Compared with Bt cotton DP99B, conventional variety CCRI 35, depending on its significantly lower K_m and C_{min}, might have a competitive edge on using limited K^+ source. But it is yet testified if susceptibility of premature senescence of DP99B was highly related to low K^+ uptake efficiency in seedling stage. Although high internal K^+ of root could affect I_{max}, K_m and C_{min}, 0.49% increment of K^+ concentration in root from 1.47% (for CCRI 35) to 1.96% (for DP99B) could not sufficiently account for bigger disparity of K_m and C_{min} between two varieties, which resulted from the facts that K^+ uptake dynamic of different varieties may vary with seedling age, and appropriate initial K^+ concentration during depletion for different varieties may be distinct.

4.3 Evaluation and prospect of K^+ uptake kinetics suitable to cotton

K^+ uptake kinetics by root has played a great role in selecting high K^+ efficient species and varieties[8], but few applications on cotton were found. Above results showed that K^+ uptake kinetics maybe suitable to select cotton varieties with high potassium efficiency, but it needs to investigate whether K^+ uptake efficiency difference among cotton varieties in seedling stage could be applied to other growth and development stages, especially to maturation stage.

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