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烟草根皮层原生质体质膜钾通道的特性研究

刘卫群[1]，王卫民[1]，石永春[1]，宋纯鹏[2]

(1.河南农业大学农学院，郑州 450002；2.河南大学生命科学学院，开封 475001)

摘 要：采用膜片钳技术对烟草根皮层原生质体质膜上的钾通道进行全细胞记录，从而深入研究烟草 K^+ 的吸收机制和调控机理。结果表明，内向钾通道在膜电压低于－40 mV 时，可以被 K^+ 激活。内向电流可以被钾通道的专一抑制剂 TEA^+ 抑制。动力学分析表明内向钾电流产生的 K^+ 表观解离常数(K_m)≈15.2 mmol/L，类似于低亲和性钾通道。该通道具有依赖于胞外 K^+ 浓度的特性，对胞外 NH_4^+、Ca^{2+}、Mg^{2+} 浓度变化反应敏感，内向 K^+ 电流可被不同程度地抑制。

关键词：膜片钳；K^+ 通道；烟草；全细胞；吸收机制

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The Character of Inward K^+-channels in the Plasma Membrane from Tobacco Root Cortex Protoplasts

LIU Wei-Qun[1], WANG Wei-Min[1], SHI Yong-Chun[1], SONG Chun-Peng[2]

(1. *Agronomy College of Henan Agricultural University*, Zhengzhou 450002, China;
2. *College of Life Sciences, Henan University*, Kaifeng 475001, China)

Abstract: To understand the mechanism of K^+ uptake and the regulated factors, patch clamp whole-cell recording techniques were applied to isolated tobacco root cortex protoplasts. The results showed that the inward current were activated by K^+ into root cortex at membrane potentials more negative than −40 mV. The inward currents were strongly inhibited by the K^+-channel blocker TEA^+ kinetic analysis of the inward currents yielded an apparent K^+ equilibrium dissociation constant (K_m) of≈15.2 mmol/L, which closely correlated to the major component of low-affinity K^+ uptake. The inward K^+-channels were sensitive to NH_4^+ and NH_4^+ was acted on a binding site external to the channel pore. The inward currents were inhibited differently by Ca^{2+} and Mg^{2+}. It is suggested that the inward K^+ channels in root cortex may function as both a physiologically important mechanism for low-affinity K^+ uptake and regulators of membrane potentials.

Key words: Patch clamp; K^+-channels; Tobacco; Whole cell; Uptake

K^+ is an important macronutrient element for higher plants. K^+ transport across the plasma membrane is closely linked to diverse tissue- and cell-specific actions related to plant growth and development, such as germination, leaf movements, stomatal action, vascular transport, and enzyme homeostasis[1-3]. Detailed kinetic analyses of K^+ uptake into roots have suggested the presence of at least two separate classes of transporters[1,4-6]. The high-affinity K^+ transporter (mechanism Ⅰ) was found with a K_m of approximately 15 to 40 μmol/L, which saturates at 200 μmol/L external K^+[1,4]. This high-affinity transporter represents a major mechanism for K^+ uptake from soil. Low-affinity transporter (mechanism Ⅱ) with a K_m for K^+ of 4 to 16 mmol/L contributes to K^+ uptake at concentrations greater than 300 μmol/L K^+[1,4]. Studies have shown that the activity of the high-affinity transporter is reduced at elevated K^+ levels, and the low-affinity transporter may contribute significantly to K^+ uptake at concentrations greater than 300 μmol/L K^+[7-9]. Several molecular

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作者简介：刘卫群(1956－)，女，河北人，教授，博士生导师，从事烟草生理生化研究(E-mail:liuweiqun2004@126.com)。

mechanisms have been suggested for K^+ uptake at the root epidermis, including K^+ pumps[10], K^+/H^+ antiporters[11], and K^+/H^+ symporters[12] as high-affinity K^+ uptake mechanisms, and K^+ channels as a low-affinity K^+ uptake mechanism[13,14]. Research on root cells has revealed that inward K^+-channels provide an important mechanism for low-affinity K^+ uptake[15,16].

Tobacco is an important model plant and has been studied widely in biology and biochemistry. It is considered valuable to use the model plant of tobacco to study the mechanism of K^+ uptake and improve the character of K^+ nutrition. This will provide a background to improve the K^+ nutrition of higher plants by using genetic engineering. But the work depends on the character of K^+-channels and the regulated mechanisms. Though some studies had applied the patch clamp techniques to tobacco cells, these researches correlated only with suspended cells and mesophyll cells[17-20]. Patch clamp whole-cell recording techniques were applied to characterize the inward K^+-channels in the plasma membrane of protoplast isolated from tobacco root cortex (PTRC). This study represented infrequent attempt to isolate plasma membrane K^+-channels and correlated K^+ transport through the channels with the other important elements of nutrition in soil. The results of this study revealed that the inward K^+-channels may function as major component of the low-affinity K^+ uptake systems operating in intact roots of higher plants.

1 Materials and Methods

1.1 Plant growth

Seeds of tobacco (*Nicotiana tobacum* L. cv. rustica America) were sown in perlite and grown at 30℃/20℃ (day/night) temperatures with a 14 h photoperiod and 200 to 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PAR. RH was maintained at 60% to 80%. Plants were harvested after 3—4 weeks when roots were typically 4—6 cm long.

1.2 Protoplast preparation

Roots were briefly washed in distilled water before being removed from the plants. The following protocol was used to isolate protoplasts from the cortex. Roots from about 50 seedlings were finely chopped into pieces of 0.5 to 1 mm long in 1.5 mL of enzyme solution (2.5% cellulase [R 10 Onozuka], 0.1% macerozyme [R 10 Onozuka], 0.1% BSA [Sigma], 10 mmol/L K-glutamate, 1 mmol/L $CaCl_2$, 2 mmol/L $MgCl_2$, 5 mmol/L Mes [Sigma], pH6.0 adjusted with Tris). The chopped tissue was agitated at 28℃ in a water bath shaker (50 r/min) for 60 min. The digest was filtered through 50 μm nylon mesh. Protoplasts were collected by centrifugation (5 min, 550 r/min; 28℃) and washed twice with the standard solution. The protoplasts were then resuspended in external solution and kept on ice until required.

1.3 Electrophysiology

Whole-cell currents from protoplasts were recorded[21] at room temperature with Epc-9 using conventional patch clamp techniques. Cells were held in a following chamber of less than 0.5 mL volume allowing fast solution changes. The chamber had a thin glass base to which protoplasts adhered loosely. Electrodes were pulled from borosilicate glass capillaries and fire polished using a PC10 (Narishige, Japan) to give resistances of 10—11 MΩ in "pipette solutions" (Table 1). And Ag/AgCl reference electrode was connected to the bath via a 3 mol/L KCl/agar salt bridge. After giga-ohm seals were formed, strong suction was applied to the interior of the pipette to obtain the whole-cell configuration. Whole-cell capacitance and series resistance were partially compensated for by the amplifier. Access resistance was usually less than 30 MΩ. Analysis of data was done by pulse-8.5 software.

Table 1 Equilibrium potential for the major ions

Item	K^+	Mg^{2+}	Ca^{2+}
Pipette solution (mmol/L)	100.0	4.0	0.0001
Bath solutionm(mmol/L)	10	4.0	2.0
Equilibrium potential(mV)	−58.0	−1.5	>0

1.4 Solutions

All solutions (Table 2) were filtered (0.22 μm, Millipore) before use. The patch clamp pipette solution consisted of (mmol/L): 100 K-glutamate, 10

Hepes, 2 $MgCl_2$, 2 EGTA, 2 ATP-Mg, pH7.2 with BTP, replenished with Dsorbitol to an osmolarity of 385 mOsm/kg. The stand bath solution consisted of (mmol/L): 10 K-glutamate, 1 $CaCl_2$, 2 $MgCl_2$, 5 MES, pH6.0 with KOH, replenished with Dsorbitol to an osmolarity of 300 mOsm/kg. The bath solution consisted of (mmol/L) 10 K-glutamate, 2 $CaCl_2$, 4 $MgCl_2$, 5 MES, pH6.0 with Tris, replenished with D-sorbitol to an osmolarity of 345 mOsm/kg. Also, when varying the external K^+, TEA^+, NH_4^+, Cl^-, Ca^{2+} and Mg^{2+} concentrations, the solutions contained the indicated amount of K-glutamate, TEACl, NH_4Cl, $CaCl_2$ and $MgCl_2$.

Table 2 The components of solution (mmol/L)

Item	Glu-K	$CaCl_2$	$MgCl_2$	Hepes	Mes	ATP-Mg	EGTA	pH	Osmotic concentration (mOsm/kg)
Stand bath solution	10	1	2		5			6.0	320
Bath solution	10	2	4		5			6.0	350
Pipette solution	100	2	2	10		2	2	7.2	400

Abbreviations used: BTP, 1, 3-bis [tris (hydroxymethyl)-methylamino] propane; Hepes, 4-(2-hydroxyethyl)-1-piperazineethane sulphonic acid; Mes, 2-(N-morpholino) ethane sulphonic acid; Tris, tris (hydroxymethyl) aminomethane; BSA, bovine serum albumin; EGTA, ethyleneglycol-bis (aminoethylether)-N, N'-tetraacetic acid.

2 Results

With 10 mmol/L K^+ in the bath solution and 100 mmol/L K^+ in the pipette solution, the whole-cell resistance was 1.2 ± 0.2 GΩ (mean ± SD; 12 cells). The value of the diameter of the protoplasts used in this study was 12 ± 3 μm (mean ± SD; 30 cells). Protoplasts of similar size (approximately 12 μm in diameter) were chosen for the experiments.

2.1 Identification of the time-dependent inward currents

Protoplasts were isolated from tobacco root cortex and used for patch-clamp measurements in the whole-cell configuration (Fig. 1). Figure 2: a shows typical recordings of activation of inward K^+-currents in the plasma membrane upon hyperpolarizing pulse potentials ranging from −170 mV to 10 mV. Tail-current protocols were used to determine the major ion responsible for the time-dependent inward current. The reversal potentials (E_{rev}) of the tail currents were determined as described in Figure 2: c. The reversal potential of the inward currents for protoplasts in the bath solution was -40 ± 4.2 mV (n=5). This is much closer to the equilibrium potential for K^+ in the solution (E_k = −58 mV). The K^+-channel blocker TEA^+[22,23], at a concentration of 10 mmol/L in the bath solution reduced the inward currents by 67.5% with 2 minutes relative to control in −170 mV (Fig. 3). These results suggested that the inward currents were major K^+ influx.

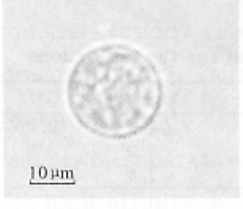
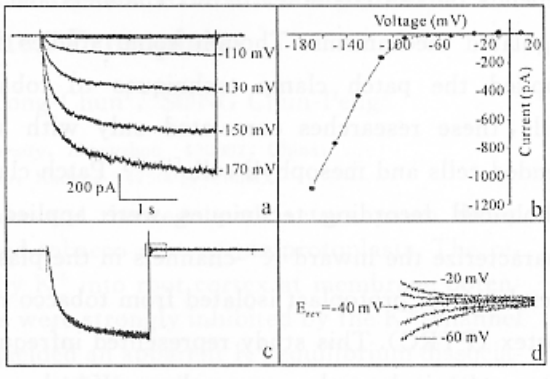


Fig. 1 Protoplasts isolated from the cortex of a young tobacco root cortex

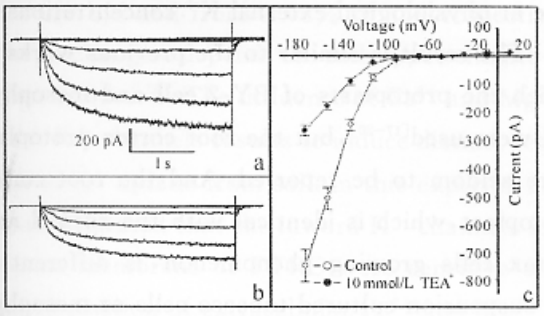


The bath solution was used and the membrane potential was held at −58 mV. a, Plasma membrane currents resulting from voltage pulses ranging from −170 mV to 10 mV (in 20 mV steps at intervals of 5 seconds). b, Current-voltage (I-V) relationship of the time-dependent component of the currents shown in a. c, Tail currents from a cortical protoplast resulting from voltage pulses ranging from −150 mV to 0 mV. For clarity, only currents responses from voltage pulses ranging from −60 mV to −20 mV are shown. Holding potential was −58 mV. E_{rev} = −40 mV. d, Enlargement of the boxed area in c

Fig. 2 Recordings of whole-cell inward currents across the plasma membrane of PTRC

2.2 Sensitivity to K^+

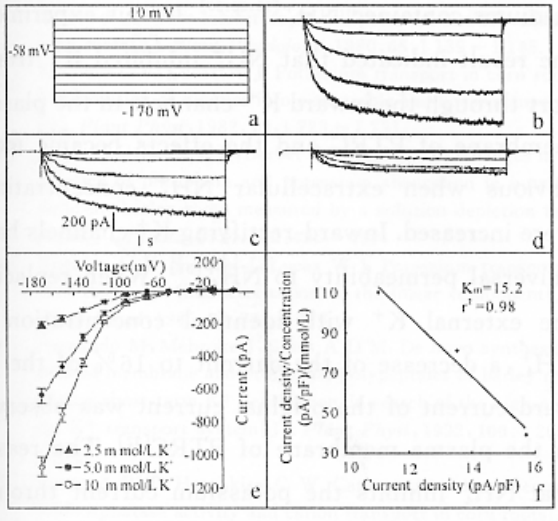
The phenomenon that the inward currents de-



a, Before addition of TEA^+. b, 2 min after adding 10 mmol/L extracellular TEA^+. c, Current-voltage relationship of time-dependent in varying concentrations of extracellular TEA^+

Fig. 3 The effect of extracellular TEA^+ on the inward currents from PTRC

pending on the external K^+ concentrations was used to explain the kinetic parameters of K^+ uptake. Figure 4:b,c,d showed that the magnitude of the inward currents increased as the extracellular K^+ concentration increased, but the saturation of the inward currents voltage decreased as the extracellular K^+ concentration decreased. Analysis of the current-voltage relationship of the currents, which obtained from tobacco root cortex exposed to varying external K^+ concentrations, indicated that the activation potential of inward currents shifted to more negative values with decreasing K^+ concentrations (Fig. 4:e).



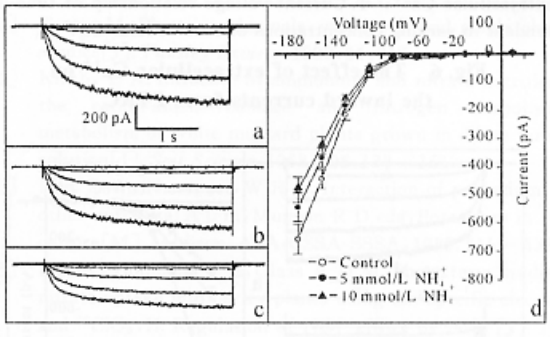
a, Pulses were applied ranging from −170 to 10 mV with 20 mV steps. b, Currents in 10 mmol/L K^+. c, Currents in 5 mmol/L K^+. d, Currents in 2.5 mmol/L K^+. e, Current-voltage relationship of time-dependent in varying extracellular K^+ concentrations. f, Michaelis-Menten function in extracellular K^+ concentrations ranging from 2.5 to 10 mmol/L

Fig. 4 The effects of changes in extracellular K^+ concentrations on the currents from PTRC

Numerous studies have shown that K^+ uptake by higher plant cells can be described by dual Michaelis-Menten kinetics[1,4]. Fig 4:f shows that the dependence of the K^+ current density on the extracellular K^+ concentrations was well described by Michaelis-Menten kinetics, with a K_m of 15.2 mmol/L, resembling kinetic properties of major low-affinity K^+ uptake component in higher plant cells[1,4].

2.3 Sensitivity to NH_4^+

When the stand bath solution contained the indicated 5 mmol/L NH_4Cl, the inward current was reduced by 17.1% relative to the current observed with control in −170 mV (Fig. 5:a,b,d). And the current was further reduced by 26.6% with 10 mmol/L extracellular NH_4Cl (Fig. 5:a,c,d). The above-mentioned changes in the inward currents showed that NH_4^+ inhibited the activity of inward K^+-channels and the effects became more obvious with extracellular NH_4^+ concentrations improved.



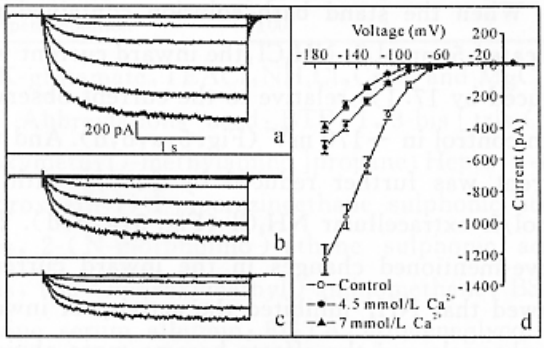
a, Before addition of NH_4^+. b, 5 min after adding 5 mmol/L extracellular NH_4^+. c, 5 min after adding another 5 mmol/L extracellular NH_4^+. d, Current-voltage relationship of time-dependent in varying concentrations of extracellular NH_4^+

Fig. 5 The effect of extracellular NH_4^+ on the inward currents from PTRC

2.4 Sensitivity to Ca^{2+} and Mg^{2+}

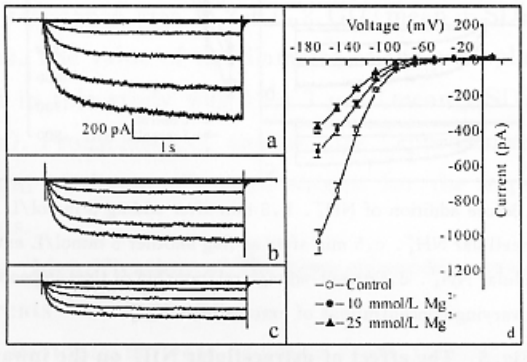
When the bath solution contained 4.5 mmol/L Ca^{2+} (2.5 mmol/L Ca^{2+} added), the inward current was reduced by 57.7% relative to the current observed with control (2 mmol/L Ca^{2+}) in −170 mV (Fig. 6:a,b,d). The current was further reduced by 68% with another 2.5 mmol/L extracellular Ca^{2+} added (Fig. 6:a,c,d). The sensitivity of the inward currents to extracellular Mg^{2+} was also investigated. Extracellular 14 mmol/L Mg^{2+}

(10 mmol/L Mg^{2+} added) reduced the inward current by 50.3% relative to the current observed in the control (4 mmol/L Mg^{2+}) in −170 mV (Fig. 7:a,b,d). And the current was further reduced by 68% with another 15 mmol/L extracellular Mg^{2+} added (Fig. 7:a,c,d). To summarize, the experiment demonstrated that the activity of inward K^+-channels was inhibited by Ca^{2+} and Mg^{2+} and the effects become more obvious with extracellular Ca^{2+} and Mg^{2+} concentrations increased.



a, Before addition of Ca^{2+}. b, 10 min after adding 2.5 mmol/L extracellular Ca^{2+}. c, 10 min after adding another 2.5 mmol/L extracellular Ca^{2+}. d, Current-voltage relationship of time-dependent in varying concentrations of extracellular Ca^{2+}

Fig. 6 The effect of extracellular Ca^{2+} on the inward currents from PTRC



a, Before addition of Mg^{2+}. b, 10 min after adding 10 mmol/L extracellular Mg^{2+}. c, 10 min after adding another 15 mmol/L extracellular Mg^{2+}. c, Current-voltage relationship of time-dependent in varying concentrations of extracellular Mg^{2+}

Fig. 7 The effect of extracellular Mg^{2+} on the inward currents from PTRC

3 Discussion

Here, the data of this initial voltage-clamp analysis of root cortex demonstrated that the inward K^+-channels constituted a major K^+ transport mechanism in root cortex and that the K^+-channels provided a means for low-affinity K^+ uptake at physiological external K^+ concentrations.

The result is similar to the previous works in which the protoplasts of BY-2 cell and mesophyll cell were used[17-20], but the root cortex protoplast were seldom to be reported. And the root cortex protoplast, which is identical with the normal root cortex cells growing phenomenon, is different to the suspension cultured tobacco cells or mesophyll cells. The result showed the extra-cellular K^+ concentration lowed in conjunction with the decreasing of K^+ inward in PTRC. Obviously, extra-cellular lower K^+ concentrations don't contribute to low-affinity K^+ uptake.

There were similarities between NH_4^+ and K^+, for example in the charge of ions, the diameter of hydration cations and the effect on membrane potential. These characters cause NH_4^+ and K^+ to compete in biological activity such as the interaction effect in plant passive uptake and the influence on the component of plant tissue in the experiment on long-time uptake[24,25], the impact on the speeds of inward ion transport and outward ion transport in the experiment on short-time absorption[13,26-29]. The present studies indicated that NH_4^+ could significantly reduce the quantity of K^+ uptake when mediums contained NH_4^+[13,26,30]. In this experiment the result indicated that NH_4^+ inhibited K^+ transport through the inward K^+-channels in the plasma membrane of PTRC and the effects became more obvious when extracellular NH_4^+ concentrations were increased. Inward-rectifying K^+ channels have universal permeability to NH_4^+[31]. When replacing the external K^+ with identical concentration of NH_4^+, a decrease of the current to 16% of the inward current of the original current was observed in the plasma membrane of PTRC[32]. The reason that NH_4^+ inhibits the potassium current through the inward channels is possibly that NH_4^+ acts on a binding site external to the channel pore, which in some way affects the permeability of the channel.

Both Ca^{2+} and Mg^{2+} are necessary macronutrients for higher plants and they exist widely in soil. The content of Ca^{2+} and Mg^{2+} in soil ranges from

trace to 4% and from 0.05% to 4%, respectively. These changes depend on soil type, climate and other factors[33]. The influence of Ca^{2+} and Mg^{2+} on plant K^+-channels results to induce distinct regulation of K^+ uptake from soil. In this experiment the result indicated that the inward K^+-channels in the plasma membrane of PTRC were sensitive to the transformation of extracellular Ca^{2+} and Mg^{2+} concentrations. Ca^{2+} and Mg^{2+} inhibited potassium transport through inward K^+-channels and the effects became more obvious when extracellular Ca^{2+} and Mg^{2+} concentrations were increased. So Ca^{2+} and Mg^{2+} reduce K^+ uptake from soil when the mediums have high concentrations of Ca^{2+} and Mg^{2+}.

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References:

[1] Epstein E. Kinetics of ion transport and the carrier concept [A]. In: Lüttge U, Pitman M G eds, Transport in Plants (Vol IIB) [M]. Heidelberg: Springer, 1976. 70 – 94.

[2] Clarkson D T, Hanson J B. The mineral nutrition of higher plants[J]. *Ann R Plant*, 1980, **31**: 289 – 298.

[3] Glass A D M. Plant Nutrition: An Introduction to Current Concepts [M]. Boston: Jones and Bartlett Publishers, 1989. 163.

[4] Epstein E, Rains D W, Elzam O E. Resolution of dual mechanism of potassium absorption by barley roots[J]. *Proc Nat Acad Sci USA*, 1963, **49**: 684 – 692.

[5] Cheeseman J M, LaFayette P R, Gronewald J W, Hanson J B. Effect of ATPase inhibitors on cell potential and K^+ influx in corn roots[J]. *Plant Physiology*, 1980, **65**: 1 139 – 1 145.

[6] Kochian L V, Lucas W J. Potassium transport in corn roots. I. Resolution of kinetics into a saturable and linear component [J]. *Plant Physl*, 1982, **70**: 1 723 – 1 731.

[7] Drew M C, Saker L R, Barber S A, Jenkins W. Changes in the kinetics of phosphate and potassium absorption in nutrient-deficient barley roots measured by a solution-depletion technique[J]. *Planta*, 1984, **160**: 490 – 499.

[8] Kochian L V, Xin-Zhi J, Lucas W J. Potassium transport in corn roots. IV. Characterization of the linear component[J]. *Plant Physl*, 1985, **79**: 771 – 776.

[9] Fernando M, Mehroke J, Glass A D M. De novo synthesis of plasma membrane and protoplast polypeptides of barley roots during short-term K^+ deprivation: in search of the high-affinity K^+ transport system[J]. *Plant Physl*, 1992, **100**: 1 269 – 1 276.

[10] Leonard R T, Hotchkiss C W. Cation-stimulated adenosine triphosphatase activity and cation transport in corn roots[J]. *Plant Physl*, 1976, **58**: 331 – 335.

[11] Poole R J. Energy coupling for membrane transport[J]. *Ann Rev Plant Physiol*, 1978, **29**: 437 – 460.

[12] Rodriguez-Navarro A, Blatt M R, Slayman C L. A potassium-proton symport in *Neurospora crassa*[J]. *The Journal of General Physiology*, 1986, **87**: 647 – 674.

[13] Scherer H W, MacKown C T, Leggett J E. Potassium-ammonium uptake interaction in tobacco seedlings[J]. *J Exp Bot*, 1984, **35**: 1 060 – 1 070.

[14] Schroeder J I, Raschke K, Neher E. Voltage dependence of K^+ channels in guard-cell protoplasts[J]. *Proc Nat Acad Sci USA*, 1987, **84**: 4 108 – 4 112.

[15] Roberts S K, Tester M. Inward and outward K^+-selective currents in the plasma membrane of protoplasts from maize root cortex and stele[J]. *Plant J*, 1995, **8**: 811 – 825.

[16] Gassmann W, Schroeder J I. Inward-rectifying K^+ channels in root hairs of wheat[J]. *Plant Physl*, 1994, **105**: 1 399 – 1 408.

[17] Van duijn B, Ypey D L, Lbbenga K R. Whole-cell K^+ currents across the plasma membrane of tobacco protoplasts from cell-suspension cultures [J]. *Plant Physl*, 1993, **101**: 81 – 88.

[18] Van duijn B. Hodgkin-Huxley analysis of whole-cell outward rectifying K^+-currents in protoplasts from tobacco cell suspension cultures [J]. *Journal of Membrane Biology*, 1993, **132**: 77 – 85.

[19] Blom-Zandstra M, Koot H, Hattum J V, Vogelzang S A. Transient light-induced changes in ion channel and proton pump activities in the plasma membrane of tobacco mesophyll protoplasts[J]. *J Exp Bot*, 1997, **314**: 1 623 – 1 630.

[20] Stoeckel H, Takeda K. Plasmalemmal voletage-activated K^+ currents in protoplasts from tobacco BY-2 cells: possible regulation by action microfilaments [J]. *Protoplasma*, 2002, **220**: 79 – 87.

[21] Hamill O P, Marty A, Neher E, Sakmann B, Sigworth F J. Improved patch clamp techniques for high resolution current recording from cells and cell-free membrane patches [J]. *Pflueg Arch*, 1981, **391**: 85 – 100.

[22] Bentrup F W. Potassium ion channels in the plasmalemma [J]. *Physiol Plant*, 1990, **79**: 705 – 711.

[23] Tester M. Plant ion channels: whole-cell and single channel studies[J]. *Tansley Review*, 1990, **21**: 317.

[24] Kirkby E A. Influence of ammonium and nitrate nutrition on the cation-anion balance and nitrogen carbohydrate metabolism of white mustard plants grown in dilute nutrient solution[J]. *Soil Science*, 1968, **105**: 133 – 151.

[25] Dibb D W, Thompson W R Jr. Interaction of potassium with other nutrients[A]. In: Munson R D eds, Potassium in Agriculture[M]. Madison: ASA-CSSA-SSSA, 1985. 515 – 533.

[26] Deane-Drummond C E, Glass A D M. Short term studies of nitrate uptake into barley plants using ion-specific electrodes and $^{36}ClO_3$, II. Regulation of NO_3^- efflux by NH_4^+ [J]. *Plant Physl*, 1983, **73**: 105 – 111.

[27] Pettersson S. Effects of nitrate on influx, efflux and translocation of potassium in young sunflower plants [J]. *Physiol Plant*, 1984, **61**: 663 – 669.

[28] Lee R B, Rudge K A. Effects of nitrogen deficiency on the absorption of nitrate and ammonium by barley plants[J]. *Annals of Botany*, 1986, **57**: 471 – 486.

[29] Vale F R, Jackson W A, Volk R J. Nitrogen-stimulated potassium influx into maize roots, differential response of components resistant and sensitive to ambient ammonium[J]. *Plant Cell Environ*, 1988, **11**: 493 – 450.

[30] Wang M Y, Siddiqi M Y, Glass A D M. Interactions between K^+ and NH_4^+: Effects on ion uptake by rice roots[J]. *Plant Cell Environ*, 1996, **19**: 1 037 – 1 046.

[31] White P J. Cation channels in the plasma membrane of rye roots[J]. *J Exp Bot*, 1997, **48**: 499 – 514.

[32] Bregane M, Carpaneto F. Effects of mono- and multi-valent cations on the inward-rectifying potassium channel in isolated protoplasts from maize roots[J]. *European Biophysics Journal*, 1997, **26**: 381 – 391.

[33] Tisdale S L. Soil Fertility and Fertilizer [M]. 3rd ed, New York: Edition Macmillan Publishing Co. Inc, 1984. 351.