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**The Proceedings of the International Plant Nutrition Colloquium
XVI**

Title

Ammonium Effects on Nitrate Uptake by Roots of Upland and Paddy Rice Seedlings Related to Transmembrane Potential Differences

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Publication Date

2009-04-15

Peer reviewed

Introduction

Water shortage is increasingly limiting the luxury use of water in rice cultivation and water-saving cultivation is developing as a regime to realize sustainable use of water resource in China (Xu et al, 2008). Under the cultivation condition of intermittent irrigation or even dryland, the proportion of NO_3^- -N in soil available nitrogen increases, and NO_3^- -N even becomes the dominant form instead of NH_4^+ -N which is traditionally assumed as the preferred nitrogen source for paddy rice (Shi et al, 2002). Exposure of plant roots to NH_4^+ inhibits nitrate uptake, while NO_3^- in medium has little effects on NH_4^+ uptake (Feng et al, 2003; Wang et al, 2003). Therefore, NO_3^- uptake characteristic of rice roots and NH_4^+ effects on it is a neglectless aspect for selecting nitrogen efficient rice genotypes and studying the mechanism of nitrogen nutrient under the water-saving condition.

NH_4^+ influences NO_3^- uptake probably through the feedback regulation of NO_3^- metabolism (Orsel et al, 2002; Zhuo et al, 1999), or through the direct effect on the plasma membrane (Ayling, 1993; Ullrich et al, 1984). The cotransport of NO_3^- and proton involves H^+ -ATPase, proton transmembrane gradient, transmembrane potential difference and other membrane characteristics (Yin et al, 2005; Crawford and Glass, 1998; McClure et al, 1990), therefore the effect of NH_4^+ on NO_3^- uptake also includes these aspects. Feng et al (2004) studied the relationship between NO_3^- uptake and H^+ -ATPase activity of upland rice and paddy rice and pointed out upland rice had stronger capability of nitrate uptake than paddy rice probably for its higher H^+ -ATPase activity in plasma membrane. Transmembrane potential difference directly relates with the ion transport and reflects plasma membrane ATPase activity in a sense, while electrophysiological characteristics of nitrate uptake by upland rice has seldom been touched.

In this paper, we explored the response of transmembrane potential of upland rice and paddy rice to NO_3^- supply and the effects of NH_4^+ on it. In order to discuss the relation of nitrate uptake and transmembrane potentials differences, the NO_3^- uptake kinetics of upland rice and paddy rice and effects of NH_4^+ on it was followed.

1 Materials and Methods

1.1 Plant material

Three typical rice (*Oryza sativa*) species were applied, Brazilian upland rice, Wuyujing 3 (japonica) and Yangdao 6 (indica). Rice seeds were germinated in dark at 35°C and then transferred to a mesh in 1/2 strength IRRI rice nutrition solution and grown at $28\pm 1^\circ\text{C}$, 250~300 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, 14 h daylight and 80 % relative humidity. pH was adjusted twice everyday to 6.0 with HCl or NaOH. The culture solution was aerate intermittently for 2 h and changed every three days. 20-day-old seedlings were transplanted to the nitrogen-free nutrient solution and 3 days later nitrate uptake experiment and electrophysiological measurement were carried out.

1.2 Electrophysiological measurement

Roots of whole rice seedlings were introduced into a Plexiglass chamber that was perfused with a basic buffer solution consisting of 5 $\text{mmol}\cdot\text{L}^{-1}$ MES, 0.5 $\text{mmol}\cdot\text{L}^{-1}$ CaCl_2 and 0.05 $\text{mmol}\cdot\text{L}^{-1}$ KCl, adjusted to pH6.0 with NaOH. Transmembrane electrical potentials were measured with glass microelectrodes filled with 100 $\text{mmol}\cdot\text{L}^{-1}$ KCl and reference salt bridges filled with 100 $\text{mmol}\cdot\text{L}^{-1}$ KCl in 2% agar, connected via Ag/AgCl electrodes with an

electrometer amplifier (SED, Stellenbosch University, South Africa) and a line recorder. The reference electrode was kept in the vicinity of the root, and the micropipette was inserted with a micromanipulator into the root at the beginning of the root hair zone (10~20mm from the tip). After the root cells attained a resting potential with the basic buffer solution, the perfusion solution was changed to the treatment buffer solutions, in which contained 0.1 or 1.0 mmol·L⁻¹ NO₃⁻ with or without 1.0 mmol·L⁻¹ NH₄⁺, and the concentrations of MES, K⁺ and Ca²⁺ were same as in the basic solution, and pH maintained 6.0. NO₃⁻ and NH₄⁺ was separately provided with the form of Ca(NO₃)₂ and NH₄Cl. In order to keep the same Ca²⁺ concentration as in the basic solution, extra CaCl₂ was added into the treatment solution containing 0.1mmol·L⁻¹ NO₃⁻.

1.3 Nitrate uptake kinetics experiments

A series solutions were prepared for nitrate uptake with Ca(NO₃)₂ and NO₃⁻ concentrations were respectively 0.05, 0.1, 0.2, 0.4, 0.6, 1.0 and 2.0 mmol·L⁻¹. pH of all the NO₃⁻ uptake solution was 6.0. To study the effects of NH₄⁺ on NO₃⁻ uptake kinetics, 0.5 mmol · L⁻¹ (NH₄)₂SO₄ was added to the NO₃⁻ uptake solutions. 10 rice seedlings were selected and their roots were immersed in 50 mL uptake solution for 2 h at 28±1□, 250~300 μmol·m²·s⁻¹, and 80 % relative humidity. Then the fresh roots were cut and weighted. NO₃⁻ concentrations in uptake solution was measured with the method of ultraviolet spectrophotometer. According to the differences of NO₃⁻ concentrations before and after uptake, net uptake rate of NO₃⁻ was calculated. Then Michaelis-Menten equation was applied to get V_{max} (the maximum uptake rate) and K_m (the apparent Michaelis-Menten constant).

2. Results

2.1 Response of membrane potential difference to nitrate

Upon addition of nitrate, an immediate decrease of the transmembrane potential difference (Em) of rice root cells was observed (Fig 1). Depolarization of upland rice roots reached 18.8mV (0.1 mmol·L⁻¹ NO₃⁻) and 25.1mV (1.0 mmol·L⁻¹NO₃⁻), which were significantly higher than that of two paddy rice (Table 1). This initial depolarization was followed by a slow repolarization. Membrane potential recovered to initial level 10 to 15 minutes after 0.1mmol·L⁻¹ nitrate was supplied and even hyperpolarization was observed in some cases, while 1.0 mmol·L⁻¹ nitrate was difficult to lower Em to initial values except nitrate free solution was added.

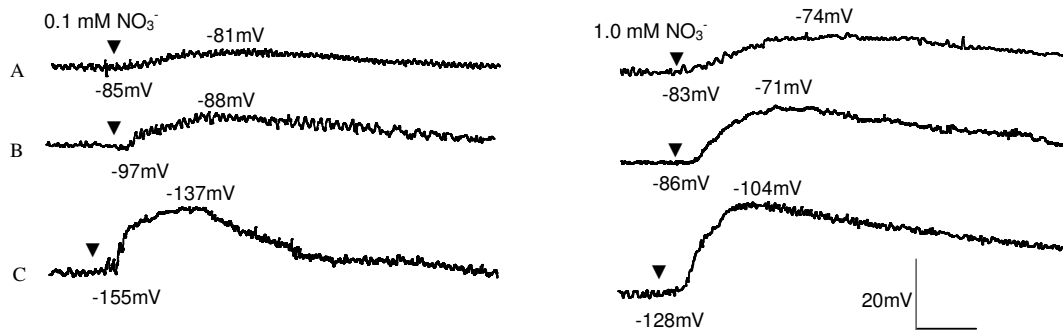


Fig.1 Representative recording curves to demonstrate the response of transmembrane potential differences in epidermal cells of rice seedling roots to 0.1 mmol·L⁻¹ (left) or 1.0 mmol·L⁻¹ NO₃⁻ (right). The arrow▼ means the addition of a treatment buffer solution. A, Wuyujing 3; B, Yangdao 6; C, Brazilian upland rice.

Table 1 Resting membrane potential of rice and depolarization degrees induced by different perfusion buffers.

Species	Resting membrane potential (mV)	Depolarization degree (mV)			
		0.1mM NO ₃ ⁻	0.1mM NO ₃ ⁻ and 1.0mM NH ₄ ⁺	1.0mM NO ₃ ⁻	1.0 mM NO ₃ ⁻ and 1.0mM NH ₄ ⁺
Wuyujing3	-78.2 ± 4.6 b (n = 40)	3.9 ± 0.8 c (n = 9)	15.7 ± 1.1 b (n = 13)	7.8 ± 1.3 c (n = 10)	24.3 ± 4.7 b (n = 8)
Yangdao6	-84.5 ± 5.1 b (n = 35)	9.8 ± 1.3 b (n = 8)	17.3 ± 2.5 b (n = 10)	14.4 ± 2.1 b (n = 9)	27.1 ± 3.6 b (n = 8)
Brazilian upland rice	-136.8 ± 9.2 a (n = 47)	18.8 ± 2.3 a (n = 12)	38.4 ± 5.2 a (n = 16)	25.1 ± 2.9 a (n = 8)	42.6 ± 5.5 a (n = 11)

The small letter represents the significance of differences among rice species. Numbers in the parentheses are the measurement times.

2.2 Response of Em to coexistence of nitrate and ammonium

Coexistence of NO₃⁻ and NH₄⁺ aggravated depolarization and shortened repolarization (Fig 2). In some cases, no obvious repolarization was observed until perfusion solution was changed to nitrate free basic buffer. Depolarization degree of upland rice roots reached 38.4mV (0.1 mM NO₃⁻) and 42.6mV (1.0 mM NO₃⁻), much higher than that of two paddy rice (Table 1).

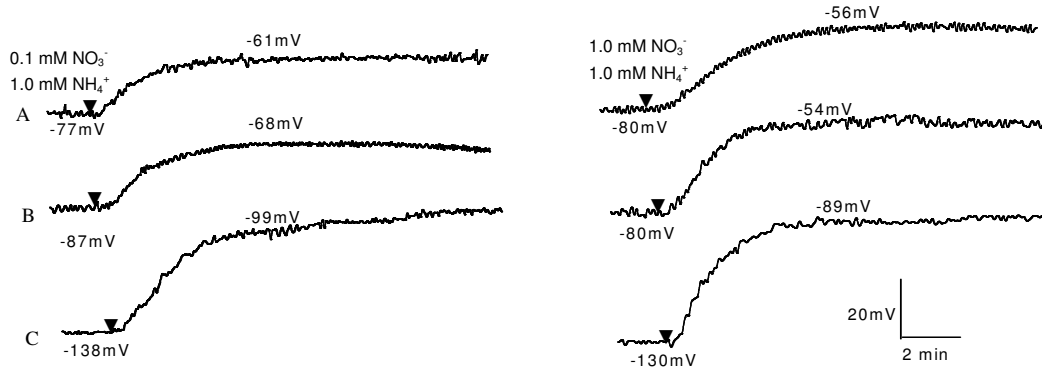


Fig.2 Representative recording curves to demonstrate the response of transmembrane potential differences in epidermal cells of rice seedling roots to simultaneous addition of NH₄⁺ and NO₃⁻. The arrow ▼ means the addition of a treatment buffer solution. A, Wuyujing 3; B, Yangdao 6; C, Brazilian upland rice. Left, 1.0 mM NH₄⁺ and 0.1 mM NO₃⁻; right, 1.0 mM NH₄⁺ and 1.0 mM NO₃⁻.

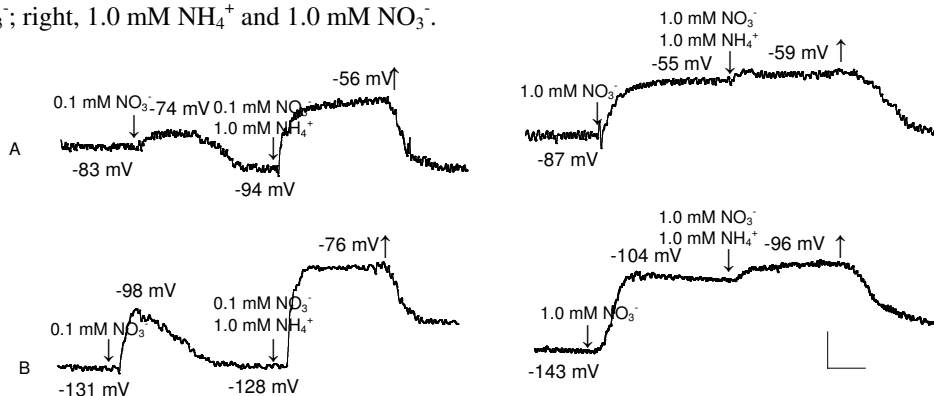


Fig.3 Representative recording curves to demonstrate the response of transmembrane potential differences in epidermal cells of rice seedling roots to first addition of NO₃⁻ or NH₄⁺ then simultaneous addition of NO₃⁻ and NH₄⁺. The downward arrow ↓ means the addition of a treatment buffer solution, and the upward arrow ↑ means the addition of the basic solution without nitrate and ammonium. A, Yangdao 6; B, Brazilian upland rice.

The response of Em to coexistence of NO_3^- and NH_4^+ was related to the forms of original nitrogen source (Fig 3). First addition of NH_4^+ caused a steady depolarization, and therefore following simultaneous supplementation of NH_4^+ and NO_3^- only slightly decreased Em; first addition of NO_3^- caused a transient depolarization, which was followed by a slow repolarization, even ultimate hyperpolarization, and consequently, following simultaneously addition of both nitrogen cause obvious depolarization.

2.3 Kinetics of nitrate uptake and influences of ammonium

When NO_3^- as the sole nitrogen form, V_{\max} of Brazilian upland rice was 1.8 times higher than that of other two paddy rice, and no significant difference existed between the two paddy rice (Fig 4). When NO_3^- and NH_4^+ were simultaneously supplied, V_{\max} of Wuyujing 3 decreased 63%, while that of other two rice only slightly decreased. At either with or without NH_4^+ , K_m of Wuyujing 3 was higher than that of Brazilian upland rice and Yangdao 6. Coexistence of NH_4^+ with NO_3^- obviously increased K_m of Wuyujing 3 and Yangdao 6, while only slightly increased that of Brazilian upland rice.

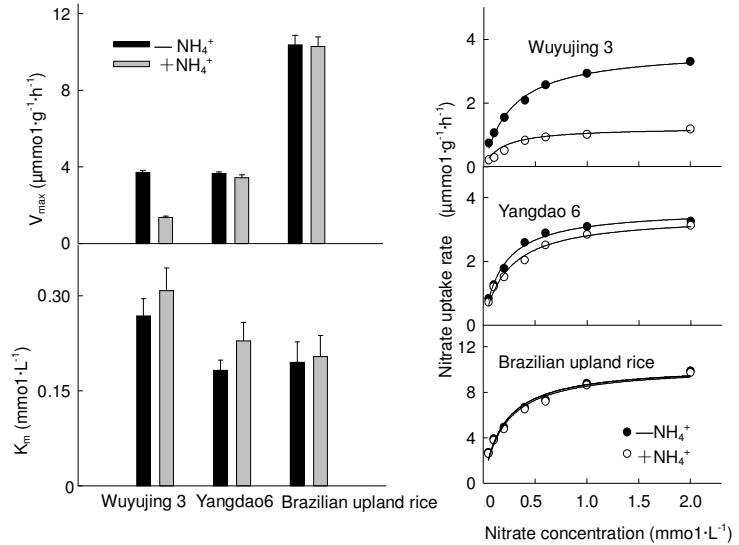


Fig.4 Kinetics of nitrate uptake by roots of different rice species

3. Discussion

Transmembrane electrical potential presented polarized status for the selective permeability of membrane to charged ions, which was related to plant variety, species, organelle, tissue, circumstances, et al (Miller et al, 2001). Glass microelectrode technique was the efficient means to study ion transport mechanisms (Miller and Wells, 2006). Depolarization of membrane potential has close relation with NO_3^- transport (Miller et al, 2001; Miller & Smith, 1996; Glass et al, 1992; Ullrich & Novacky, 1981). NO_3^- induced stronger depolarization of Brazilian upland rice than that of paddy rice (Fig 1), and higher NO_3^- uptake rate of the former than the later (Fig 4). That is to say, differences of membrane electrical potential was accorded with the sensitivity of roots to NO_3^- and capacity of NO_3^- uptake.

Membrane electrical potential can vary between certain limits, for it plays an important role in cellular signal transduction and maintains close ties with H^+ -ATPase. After first depolarization of membrane potential, nitrate-treated cells always tended to recover to their original status. Depolarization resulted from the activated NO_3^- transporter and cotransport of NO_3^- and proton (McClure et al, 1990), while following repolarization and even hyperpolarization was attributed

to the activated H^+ -ATPase, which was stimulated by depolarization (McClure et al, 1990). Hyperpolarization even occurred at low NO_3^- concentration, and negative membrane potential is helpful for NO_3^- uptake. On the other hand, distribution of potassium has a close relation to stabilization of membrane potential (Hirsch et al, 1998; Walker et al, 1996). Depolarization is favorable to the opening of potassium outward channel, in order to recover the membrane potential to the original level.

Depolarization of E_m put NO_3^- transport at a disadvantage. NH_4^+ decreased the electrical polarized status and therefore inhibited NO_3^- uptake. Coexistence of NH_4^+ and NO_3^- caused steady depolarization, which possibly related to the effects of NH_4^+ on transporter, proton pump and potassium channel. When cation influx exceeded outflow, depolarization occurred, thus proton pump was activated to recover the initial polarized status, while if proton pump was strongly inhibited, no repolarization occurred (Miller et al, 2001; Wu et al, 2001). Di et al (2007) found rice was grown with NH_4^+ had higher H^+ -ATPase activity than that with NO_3^- . Therefore, maintenance of depolarization with the presence of NH_4^+ was impossible for the inhibition of H^+ -ATPase activity. On the other hand, Activated inward potassium channel or inhibited outward channel increases the net potassium influx, which could also caused depolarization of membrane potential. Though it is widely accepted that NH_4^+ inhibited potassium uptake (Feng et al, 2007; Spalding et al, 1999), while Szczerba et al (2008) recently with isotope tracer technique found that at low potassium concentration ammonium inhibit potassium uptake by rice and at high potassium concentration NH_4^+ stimulated it. In our experiment, potassium was as low as $0.05 \text{ mmol}\cdot\text{L}^{-1}$, therefore potassium uptake by rice was inhibited by NH_4^+ , which indicated that increase of potassium net influx was not the reason for depolarization caused by NH_4^+ . Furthermore, it was indicated that maintenance of depolarization caused by coexistence of NH_4^+ and NO_3^- was mainly because for the activation of NH_4^+ transporter, but not for the inhibition of proton pump and potassium channels.

Wang et al (1994) found NH_4^+ ($2 \mu\text{mol}\cdot\text{L}^{-1} \sim 40 \text{ mmol}\cdot\text{L}^{-1}$) caused strong depolarization in root cells of rice and repolarization occurred only when NH_4^+ was removed. In this experiment E_m depolarization caused by NH_4^+ maintained more steady and time-longer than that by NO_3^- . NO_3^- induced transient depolarization and slow repolarization, even hyperpolarization. Therefore, coexistence of NO_3^- and NH_4^+ induced strong depolarization and seldom repolarization, mainly due to the effects of NH_4^+ on membrane potential. In the same time proton pump and potassium channels were also stimulated to recover the polarized status, but the effect was weaker than depolarization caused by NH_4^+ , which could explain the phenomenon in Figure 4.

Driving force of ion transport through membrane comes from the chemical and electrical potential differences. Membrane electrical potential and the activity of H^+ -ATPase was the key to NO_3^- active transport. Resting membrane potential of epidermal cell in roots of Brazilian upland rice was much lower than that of two paddy rice species (Table 1), and therefore, the former had higher driving electrical force than the later, which possibly was the one reason that upland rice showed stronger NO_3^- uptake capacity than paddy rice. Feng et al (2004) found upland rice had higher activity of H^+ -ATPase, and further deduced that upland rice had low transmembrane potential and low heteroplastic pH. Our experiment results agreed with the opinion.

With high V_{\max} and low K_m (Fig 4), Brazilian upland rice showed stronger NO_3^- uptake capacity than Wuyujing 3 and Yangdao 6. One possible reason of the phenomenon was NO_3^- transporters in the membrane of upland rice were more active and had higher affinity to NO_3^- . Coexistence of NH_4^+ with NO_3^- had obvious influence to V_{\max} and K_m of Wuyujing 3, while only had slight influence to those of Brazilian upland rice (Fig 4). Therefore, inhibition of NH_4^+ to NO_3^- uptake was possibly related to NO_3^- transporter in membrane of roots, and the influence differed among plant species with different nitrogen utility efficiency.

Acknowledgement

This study was supported by National Basic Research Program of China (“973” Program) (Grant No. 2007CB109300) and by the program of State Key Laboratory of Soil and Sustainable Agriculture.

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