

## The Effect of pH on the Mineral Nutrient Uptake in the Rice Seedlings

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벼의 無機養分 吸收에 미치는 pH의 影響

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### ABSTRACT

Absorption pattern of potassium, calcium, phosphate and nitrate ions, and the pH change during ion-absorption at pH 3.0-11.0 by *Oryza sativa* L. were studied to investigate indirectly the evidence of H<sup>+</sup>-efflux by ATPase.

The rice seedlings which were grown either in L<sup>o</sup>-dark or in L<sup>+</sup>-sunlight, were used both in each ion-absorption to compare with one another. The uptake rate of these ions appeared to favor more in L<sup>+</sup> than in L<sup>o</sup>, over all range in pH, nearly with the same pattern.

The absorption of potassium resulted in bell shape and that of calcium increased linearly to the alkali range. The shape of phosphate-absorption showed nearly the t-distribution curve with high value in acid range and the uptake of nitrate resulted in the dual peaks, but higher in acid range.

The pH of the external solution changed from the range of 3.5-11.0 to 3.5-7.0 after 1hr-absorption, and further acidified after 3hr-absorption. It is suggested that the pH change of the external solution be affected by H<sup>+</sup>-efflux which may be caused by the ATP-hydrolysis.

### INTRODUCTION

The plants use the metabolic energy for their selective ion-transport activity of the mineral nutrients, so that they can maintain life and balanced growth. This mechanism of the selectively active transport has been well known to be the processes through enzymatic reaction to mediate ion-transport, better explained by the alternative assumption; the carrier theory or the ion pumps theory, but the latter is more effective.

According to this theory, the ion transport process is defined as both the primary process which directly causes the 'uphill' movement, for instance, Na<sup>+</sup> or H<sup>+</sup> with a chemical reaction such as the hydrolysis of ATP, and the secondary process which indirectly transports the other ions to the back-fluxes of Na<sup>+</sup> or H<sup>+</sup> down their electrochemical gradients (Hodges, 1976).

As the ion-driving force of both cation and anion influx at the plasmalemma is produced through the hydrolysis of ATP by the ATPase, the transport process driven by ATP-hydrolysis is the efflux of

H<sup>+</sup>. This gives rise to proton-motive force (pmf) or electrochemical gradient of hydrogen ion which is created by the combined effects of the difference in pH and the difference in electropotential between the solutions on either side of the membrane. Skulachev (1977) suggests that the pmf should be regarded as a biological energy source as fundamental and versatile as the high-energy phosphate bond (pmf =  $-59 \text{ pH} \Delta + \psi$ ).

The importance of the pH effect on the mineral nutrition in plants has been concerned long ago, and investigated that the pH difference between the external culture medium and the cell sap had influenced the absorption of the nutrients with relation to the metabolism (Hoagland and Broyer, 1940). And in such theories to explain the ion-regulating mechanism as Davies-Keynes theory and chemi-osmotic theory, the pH change across cell membrane had important meaning, associated with the Na<sup>+</sup>-K<sup>+</sup> pump (Bittar, 1964).

In this view, the pH factor has the most importance for life activity and more than the environmental factor like the temperature, light intensity and so forth effects the enzyme activity, especially on the cell membrane-bound proteins. In cell membrane of higher plants, ATP-dependent transport process is conducted with efflux of H<sup>+</sup>; the product of ATP-hydrolysis that the membrane-bound ATPase anticipates, and facilitates the ion-transportation of the primary process (H<sup>+</sup>-pump; Poole, 1978).

This study is purposed to investigate indirectly the reaction of H<sup>+</sup>-pump in the ion pumps theory, and examined the absorption of some mineral nutrients (potassium, calcium, phosphate, and nitrate) and the pH change of the external culture solution after the ion absorption in rice seedling.

## MATERIALS AND METHODS

### Preparation of seedlings

Rice seeds (*Oryza sativa* L., Yu-shin) provided by Crop Experimental Station, Office of Rural Development, were treated for 10 min. in a solution of 1%

sodium hypochlorite to sterilize the surface of the seeds. After washing thoroughly in the pure water, the germination process was conditioned at  $28 \pm 1^\circ\text{C}$  for 24 hrs. and then the seeds, transferred into culture plastic boxes (2 liter capacity) of seedling cotton pads, allowed to germinate for 10 days. Renewed the pad water every 24 hrs with the aerated 0.2 mM calcium sulfate, the seedlings were cultured under the different conditions; one part at  $28 \pm 1^\circ\text{C}$  in darkness (L<sup>o</sup>) and the other part at  $20 \sim 28^\circ\text{C}$  in the sunlight (L<sup>+</sup>).

### Experimental procedures

The mineral solutions were prepared with 1.0 mM potassium chloride, calcium chloride, potassium phosphate, and potassium nitrate, respectively, which were also adjusted with hydrochloric acid and sodium hydroxide so that they might have pH ranges between pH 2.0~13.0. In case of detecting the nitrate absorption, hydrochloric acid was replaced by sulfuric acid because chloride ion induced the nitrate reduction (Johnson and Ulrich, 1950). The roots of seedlings were rinsed for 20 mins in the same pH solution as each corresponding pH value to investigate just before the use, in order to prevent the disturbance of the primary pH value in mineral solution. These intact roots of 15 seedlings were then put to immerse into 50 ml beakers containing 20 ml mineral solution of the different pH value, and the beaker were placed in the water-bathed shaker at  $25 \pm 3^\circ\text{C}$  and 120 strokes/min for 1 hr or 3 hrs. At the end of the experimental time, the seedlings were removed to weight and the residual solutions were used firstly to measure the pH and then to analyze the each ion-content quantitatively. Both potassium and calcium ions were assayed by the flame photometry (Chang *et al.*, 1978), and phosphate with the ammonium molybdate method at 660 nm and nitrate with the phenoldisulfonic acid method at 410 nm were determined by the spectrophotometer. And the resulted amounts of deficit from the primary mineral solutions were calculated as the estimates of absorption.

## RESULTS

### pH change of the external solution

The pH's of the mineral solutions were measured at the end of the experimental time, in order to examine the change of them in pH during a certain period of absorption. The remarkable change resulted in the range from pH 3.5 to 10.5, while less in the extreme ranges of both acid and basic as seen in Fig. 1. The extent was more conspicuous in the basic range than in the acid.

In Fig. 1-A, which showed the change in pH after 1 hr-absorption, the range of pH 3.5~6.0 remained nearly unchanged, but that of pH 6.0~10.5 resulted in the relatively high changes, increased in proportion to the basic tendency, then to be measured the range as much as pH 6.0~7.0.

Futhermore, this acidification of the mineral solution in the basic range was found to be accelerated after 3 hr-absorption in Fig. 1-B. The primary range

of pH 4.5~9.0 turned out to be measured the range of pH 4.0~4.5.

These phenomeon were due to the low concentration of the mineral solution. The acidification was thought to be performed by the seedlings which generated H-efflux by the way of active transport through their metabolic activities. This result was conformed by Hoagland and Broyer(1936), who reported that the pH change of external culture solution after absorption upon its tropic condition, the fluctuation resulted rapidly to acidify within 3 hr-incubation in oligotrophic condition and recoverd after 21 hr-incubation, but vice versa in eutrophic condition.

And the changed range of pH 6.0~7.0 after 1 hr absorption is coincided with the growth optimum range of the rice(Wherry, 1964), since 1 hr is enough period of ion-absorption up to reach the steady-state(Chang *et al.*, 1978), and the optimum range of the K-stimulated ATP ase in higher plants (Hodge *et al.*, 1976), too.

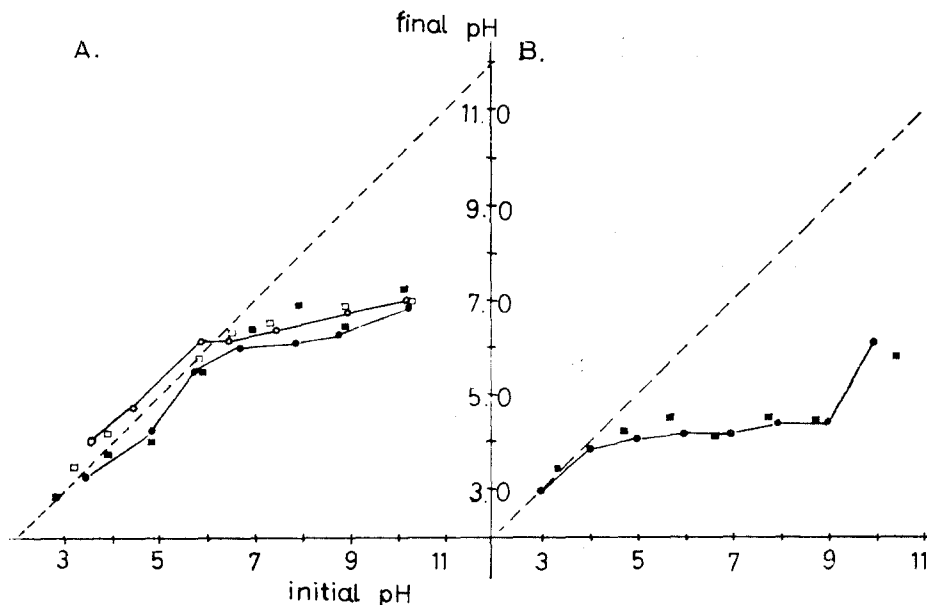


Fig. 1. The pH change in the external culture solution after absorption. A: 1 hrs. B: 3 hrs(O cation, anion uptake).

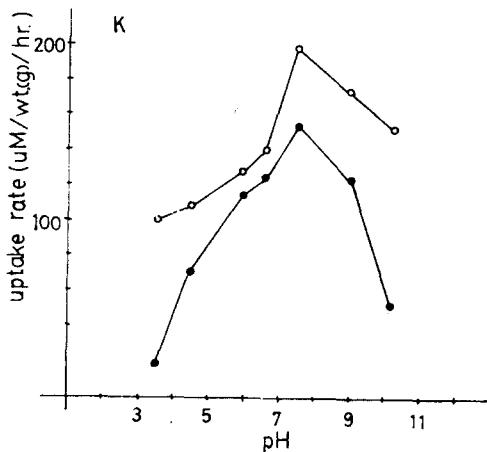


Fig. 2. The uptake rate of the potassium ion in the different pH culture sol. (○ : L<sup>+</sup>, ● : L<sup>°</sup>).

#### Absorption of potassium ion

As shown in Fig. 2, The absorption rate of potassium was investigated in the range of pH 3.0~11.0 and the maximum rate was appeared in that of pH 7.5. It was also enhanced in the basic intervals and the light-conditioned seedlings absorbed more favorably than the dark-conditioned seedlings. The comparison of the absorption rate between the acid range and the basic(L<sup>°</sup>) range revealed the enhancement in the basic, with the ratio 3 : 4 : in dark and the ratio 2 : 3 in light. On the other side, a large amount of the efflux occurred in the extreme terminal ranges damaged more in the basic, since the roots of those seedlings could hardly endure the steep H or OH concentration and hold their contents of cell sap no more, only to exudate.

The pattern of potassium absorption in rice seedling was shown to correlate with the influx exchanged antagonistically for H-efflux, which was conformed by Ratner and Jacoby(1976).

As there was no significance at the level  $0.2 < p < 0.4$  in t-test between the absorption rates calculated with fresh root wt. and that with fresh whole wt., the latter was used for the following graphs.

#### Absorption of calcium ion

Nissen(1974) reported that calcium ion was transp-

orted passively in plant cell membrane, but it was suggested that calcium, also exchanged with hydrogen ion by the plant be absorbed as carrier-mediated influx. Although calcium is dissolved differently upon pH conditions that its dissociated degree is high in acid, the absorption rate was increased with relation to the basic tendency. The correlation between pH value and the absorption rate showed highly positive correlated value,  $r = 0.82$ , and its linear regression presented  $Y = 40.4X - 108.1$  in light and  $Y = 50.3X - 254.8$  in dark. The light-conditioned seedling also absorbed more than the latter as seen in Fig. 3.

Calcium, which is known to act the inhibition of passive transport of the mineral ions in plasmalemma and to facilitate the active absorption(Cauchli and Epstein, 1970), was strangely to speak, absorbed so much in the extreme basic range. This was to study futher intensively whether it would utilized for the protective reaction of plasmalemma, or not. On the other part, in the studies on the oocyte of animal, the pH increasement from pH 6.0 to 8.0 in the external solution brought about increasing the cellular currency up to 25%, and reduced the free calcium content in cytoplasm when treated the pH decrease-ment (Robinson, 1979).

If we can apply this results to plant cell, the simiar mechanism will be proposed at the absorption

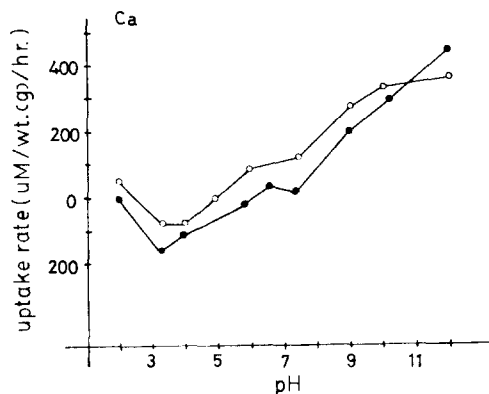


Fig. 3. The uptake rate of calcium ion in the different pH culture sol. (○ : L<sup>+</sup>, ● : L<sup>°</sup>)

of calcium ion, where the relationships between the pH increasement-dependent current change and calcium-efflux with pH decreasement may take place.

#### Absorption of phosphate ion

As seen in Fig. 4, the pattern of phosphate absorption was shown to be similar to the curve of t-distribution, the terminal curve of which rose routinely, but the absorption rate was asymmetrically higher in acid range than the basic. The maximum rate had also higher value at pH 5.8 in L<sup>+</sup>-conditioned than at pH 6.8 in the L<sup>o</sup>-conditioned in Fig. 4-A, while it had the remarkable difference after 3 hr-absorption as seen Fig. 4-B.

Since Smith and Walker(1976) suggested that the chloride or hydrocarbonate might have more important role in anion-absorption than the hydrogen, this acid-induced influx of phosphate ion could be explained as such a effect that the resultant chloride ion from the hydrochloric acid, used to regulate pH, might the anion-stimulated ATPase. Excluding this postulated effect, the external H<sup>+</sup> must cause to favor the phosphate-absorption, compared with the results by the hydroxyl ion.

On the other part, phosphate is dissolved in form of H<sub>2</sub>PO<sub>4</sub><sup>-</sup> at pH 4.0, which is available for plants, while it changes the less unavailable form of HPO<sub>4</sub><sup>2-</sup> up to 1.5%, and further basic condition makes it

undissolved form of PO<sub>4</sub><sup>3-</sup>(Larson, 1967). Hence, absorption rate of phosphate occurred more favorably in the acid range.

#### Absorption of nitrate ion

The difference between the L<sup>+</sup> conditioned and the L<sup>o</sup> conditioned was nearly distinguishable after 1 hr-absorption, but accomplished a little after 3 hr-absorption as seen in Fig. 5.

The pattern of nitrate-absorption was shown to the dual peaks, where the maximum rate appeared in acid range. It occurred both at pH 5.0 after 1 hr-absorption, and at pH 5.0 in L<sup>+</sup> condition while at pH 7.0 in L<sup>o</sup> conditioned after 3 hr-absorption.

These results could be explained that nitrate-absorption was also enhanced in the acid range, though considering the evidence, at least for the counter part. Further explanation is required in a view of the contrast suggestions, whether the anion-transport may be conducted with the redox reaction (Akinson *et al.*, 1966) or inversely with ATPase mediation(Petraglia and Poole, 1977).

### DISCUSSION

The seedlings which were precultured in sunlight, showed the higher absorption rate of these four ions over all ranges in pH than the other part, precult-

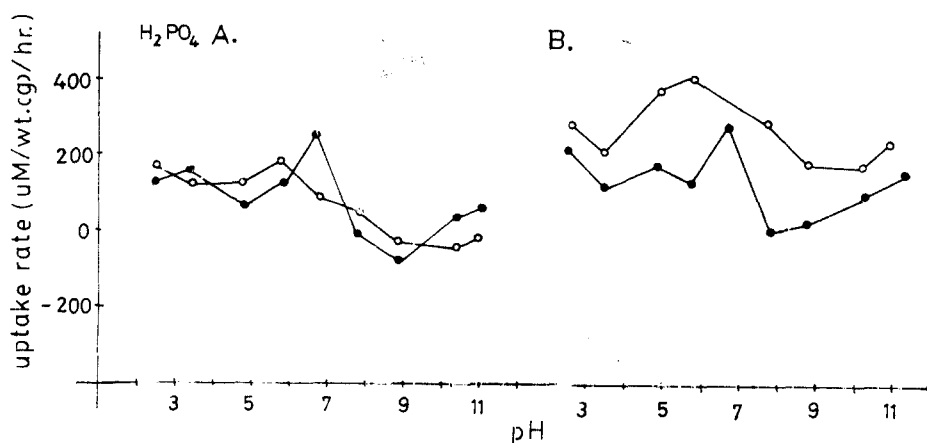


Fig. 4. The uptake rate of the phosphate ion in the different pH culture sol. (A: 1 hr, B: 3 hrs. uptake, ○ : L<sup>+</sup>, ● : L<sup>o</sup>)

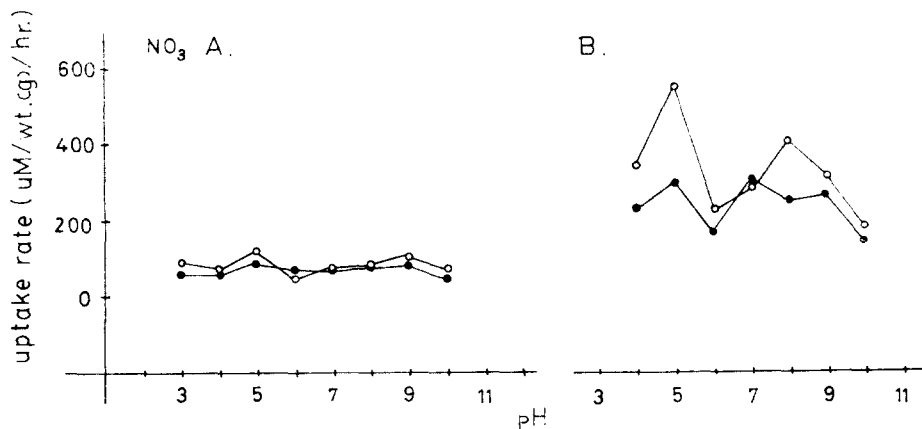


Fig. 5. The uptake rate of the nitrate ion in the different pH culture sol. (A: 1 hr, B: 3 hrs. uptake, ○ : L<sup>+</sup>, ● : L<sup>°</sup>)

ured in dark. It was thought that photosynthesis stimulated the metabolic activity as much effectively as the biosynthetic amounts of the chloroplast, produced additionally during a certain period of seedling culture, and also these results were in accord with some proposals that both cation and anion be transported actively by the reaction of ATPase (Lin and Hanson, 1976, Petraglia and Poole, 1977). So to speak, the L<sup>+</sup> condition seedlings could utilize the affluent ATP, supplied by both photophosphorylation and oxidative phosphorylation.

And the pH change of external solution after ion-absorption would be best explained as the effect of H<sup>+</sup>-efflux by ATP-hydrolysis. Otherwise, this change may be regarded as the acidification, affected by some other factors such as the respiration of the root cell, but it could not be accepted as a possible reason because the experimental condition was performed under the indirect sunlight, though not intensified up to photo-saturation, and moreover the rice roots were known to excrete O<sub>2</sub> into the surrounding medium in order to render the rhizosphere aerobic and thus to protect the rice plant from anaerobic toxins (Greenwood, 1971).

When the pattern of each ion-absorption were classified largely as cation and anion, that of cation was favorable in the basic range, while that of anion

was enhanced, on the whole, in the acid. The interaction of ion-competition for binding site, or the effect of chemical gradient for H<sup>+</sup>-efflux could explain this phenomenon. It was reported that the cation uptake was increased with counter ion as nitrate and decreased with the replaced ammonium (Rayar and Tang, 1977), and another that potassium, rubidium, and cesium ion also competed each other in the carrier binding site (Epstein and Hagen, 1952). The most alternative assumption is that H<sup>+</sup> or OH<sup>-</sup> might react as the counter ion during the ion-absorption, otherwise influence the H<sup>+</sup> chemical gradient to press or to facilitate the function of H<sup>+</sup>-pump.

In general, the plants have grown in the range of pH 4.0~8.0 in soil, and equally well at pH 4.8~7.6 in nutrient solution (Rorison, 1960), especially at pH 6.0~7.0 in rice, while the cell sap of the most plants maintain the pH range in pH 5.0~5.5. Hence, it was thought that H<sup>+</sup>-pump could be promoted under the normal pH condition of plant growth. Of course, most plants require their specific nutrients according to their different living forms and each developmental stages, but it was considered that the ion-absorption under a certain pH condition would depend upon the following problems. Firstly, the pH state of the external medium effects the change on the dissolved extent of mineral nutrients,

and further brings about so much changeable in electrochemical gradient at the plasmalemma of root cell that it may control the  $H^+$ -pump by ATP-ase to absorb the nutrients.

### 摘 要

本實驗은 유신퍼의 無機이온( $K^+$ ,  $Ca^{++}$ ,  $H_2PO_4^-$ ,  $NO_3^-$ ) 吸收에 미치는 pH의 影響을 조사한 것이다.

明·暗所에서 각각 10日間 키운 벼의 seedling을 만 일이온이 溶解된 各 pH 範圍의 배양 溶液에서 1시간, 또는 3시간 동안 吸收시키었고, 吸收後 그 溶液의 pH 變化和 各 이온의 吸收量을 分析하였다.

明·暗對照의 實驗에서  $K^+$ 의 吸收은 배양 溶液이 모두 pH 7.5일 때 最大吸收을 나타냈으며,  $Ca^{++}$ 의 吸收도 溶液의 알칼리성의 強해짐에 따라 直線的으로 增加하였다.

$H_2PO_4^-$ 의 吸收은 暗狀態( $L^+$ )에서 pH 6.8, 明狀態( $L^+$ )에서 pH 5.8일 때 最大吸收을 나타내며, 非對稱的 t-分布 모양으로 酸性範圍에서 높은 吸收을 보였고,  $NO_3^-$ 의 吸收은  $L^+$ 에서 pH 5.0일 때 最大吸收을 나타내지만, 明·暗對照에서 共히 酸·알칼리 範圍에서 촉진됨을 보였다. 吸收後 배양 溶液은 알칼리 範圍에서 모두 酸性化되었다.

이 結果로  $H^+$  濃도가 높은 酸性에서 陰이온( $H_2PO_4^-$ ,  $NO_3^-$ ) 吸收이 增加되고, 그 反對인 알칼리성에서 陽이온( $K^+$ ,  $Ca^{++}$ ) 吸收이 촉진되어  $H^+$ -pump가 외부배양 溶液의  $H^+$  濃도 勾配와 關聯性이 있음을 알 수 있다.

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