Breeding Science 44 : 383~386 (1994)

Variation in Michaelis-Menten Kinetic Parameters for Nitrate Uptake by the Young Seedlings in Rice (*Oryza sativa* L.)

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Summary

Nitrate uptake ability from low-concentration nitrate solution was evaluated based on Michaelis-Menten kinetics in rice. In 16 cultivars, including eight *japonica* and eight *indica,* apparent Km (Michaelis constant) and apparent Vmax (maximum uptake rate) ranged from 75.7 to 130.3 μM and from 4.30 to 7.17 μmol/g fresh weight/h, respectively. There was no difference in both apparent Km and apparent Vmax between *japonica* and *indica,* suggesting that there was no intraspecific difference in the gene encoding nitrate uptake carrier. However, small differences in both apparent Km and apparent Vmax were found within each subspecies. Of four mutants related to nitrate assimilation, M605, a chlorate hypersensitive mutant, showed remarkably higher apparent Km than the original cultivar, Norin 8. This fact suggests that the nitrate uptake carrier can be altered by mutation.

Key Words : *Oryza sativa* L., nitrate uptake, Michaelis-Menten kinetics, intraspecific difference, mutant.

Introduction

Investigations on the genetic aspects of plant nutrition are important for the breeding of crops with efficient use of the nutrients. In particular, improvement of nitrate uptake efficiency has a practical merit, because nitrate is a major source of nitrogen.

Recent researches have shown that nitrate uptake is the first biochemical step of the nitrate assimilation (reviewed in Wray 1986, 1988). It has been speculated that the uptake of external nitrate into cytoplasm is mediated by a carrier protein in the plasma membrane of root epidermal cell (Wray 1986), although the nitrate carrier protein has not yet been identified in crop plants. One of the evidences for existence of the nitrate carrier protein is that the nitrate uptake by plant roots can be described by Michaelis-Menten kinetics (Wray 1986). Although a gene encoding a nitrate uptake carrier has not been identified, the genetic nature of the carrier can be evaluated by two kinetic parameters, Km (Michaelis constant) and Vmax, which represent affinity of the carrier and the maximum uptake rate, respectively.

Received March 2, 1994. Accepted September 7, 1994.

Kinetic study of nitrate uptake from low-concentration nitrate solution have been reported in several higher plants, indicating that Km values depend on the genotype (Pace and McClure 1986, Konesky *et al.* 1989). Hasegawa (1992) reported that Km and Vmax of Japanese barley cultivars at 25 ℃ varied from 50.9 to 111.7 μM and from 2.24 to 4.99 μmol/g fresh weight (fw)/h, respectively, and that two parameters decreased with the drop of temperature. These facts suggest the genetic variability of nitrate uptake in higher plants and the possibility of selection for high nitrate uptake efficiency using the kinetic parameters. It is necessary for the practical breeding program to improve nitrate uptake ability in low-concentration nitrate solution.

From the fact that rice prefers ammonium as a nitrogen source but grows well under nitrate condition, we assume that rice seems to have some advantages for the study of nitrate assimilation (Hasegawa *et al.* 1991, 1992 a). Furthermore, in rice, it has been suggested that there is a difference in the nitrate assimilatory ability between two subspecies, *japonica* and *indica,* because of the difference in chlorate sensitivity between them (Morishima and Oka 1981, Ueno *et al.* 1990). It is also interesting to investigate nitrate uptake from the viewpoint of intraspecific differentiation of rice. This paper demonstrates the values of Michaelis-Menten kinetic parameters in nitrate uptake by rice seedlings for investigating the genetic ability of nitrate uptake, using 16 rice cultivars and four nitrate-assimilation related mutants.

Materials and Methods

Plant materials and seedling culture

Sixteen rice cultivars, including eight *japonica* and eight *indica,* and four mutants presented in Table 1 and 2, respectively, were used. The mutants used in this study were shown to be related to nitrate assimilation. M605 is a chlorate-hypersensitive mutant (Hasegawa *et al.* 1992 b), M819 and NR827 lack NADH-nitrate reductase activity, and M1004 is resistant to chlorate but has normal NR activity (Hasegawa *et al.* 1991, 1992 a, Ichii *et al.* 1993). M lines were isolated from the mutant lines maintained at the Institute of Radiation Breeding, Japan (Hasegawa *et al.* 1991).

Seedlings were cultured with distilled water in a growth chamber at 25±1 ℃ under natural daylight supplemented with fluorescent lamps with a 16 h photo-

period.

Measurement and analysis of nitrate uptake

Nitrate uptake was measured on the 15 th day after sowing. Prior to the experiment, seedlings were kept in an induction medium containing 100 μM KNO_3 and 400 μM $CaSO_4$ for 24h at 25 ℃. For the nitrate uptake measurement, three to nine seedlings were transferred into a small beaker with 50 ml solution containing 10 to 1,000 μM KNO_3 and 400 μM $CaSO_4$ for 2h in a biotron (Type LH-200-RD, Nippon Medical and Chemical Instruments, Japan) at 28 ℃. Endosperm of each seedling was removed just before the experiment. Two hours after treatment, nitrate concentration in the solution was measured with an ion-chromatography (2000i, Dionex, USA) adjusted with an anion analytical column (IonPac AS4A, Dionex, USA). Net nitrate uptake was calculated by the depletion from the solution. Immediately after the uptake experiment, fresh weight of the seedlings were measured. Each experiment was separately replicated three times each.

The relationship between nitrate uptake rate per fresh weight and the initial nitrate concentration of the solution was analyzed with the Michaelis-Menten equation,

$$\frac{v}{Vmax} = \frac{[S]}{[S]+Km}$$

, where v and [S] represent uptake rate and initial nitrate concentration, respectively. Vmax and Km, representing the maximum uptake rate and the Michaelis constant, respectively, were calculated from the experimental data. Km means the affinity of the nitrate uptake carrier.

Results

In all cultivars and mutant lines used in this experiment, net nitrate uptake by the roots of 15-day-old seedlings can be described by the Michaelis-Menten kinetics. In each cultivar or mutant, the uptake rate fitted very well with the Michaelis-Menten kinetics (Fig. 1).

Comparison of kinetic parameters between *japonica* and *indica*

In 16 cultivars used, apparent Km and apparent Vmax at 28℃ ranged from 75.7 to 130.3 μM and 4.30 to 7.17 μmol/g fw/h, respectively (Table 1). Averages of apparent Km and apparent Vmax in *japonica* cultivars were 99.9 μM (75.7 to 120.5 μM) and 5.92 μmol/g fw/h (4.39 to 7.17 μmol/g fw/h), respectively, and the averages in *indica* cultivars were 109.0 μM (82.2 to 130.3 μM) and 5.55 μmol/g fw/h (4.30 to 6.45 μmol/g fw/h), respectively (Fig. 2). The correlation coefficient between apparent Km and apparent Vmax of 16 cultivars is 0.604, but is not significant. However, significant correlationships between apparant Km and apparent Vmax were found in each subspecies. The correlation coefficients were 0.925 and 0.723 in *japonica* and *indica*, respectively. Fig. 2 also demonstrated that, in the cultivars which had similar level of apparent Km, *indica* cultivars tended to show lower apparent Vmax than *japonica* ones. No significant differences in the kinetic parameters between *japonica* and *indica* were observed.

In *japonica* cultivars, it seems that there is no difference in kinetic parameters between native or old cultivars and modern cultivars in this experiment. However, it is interesting that significant difference in apparent Km and apparent Vmax were found between two repre-

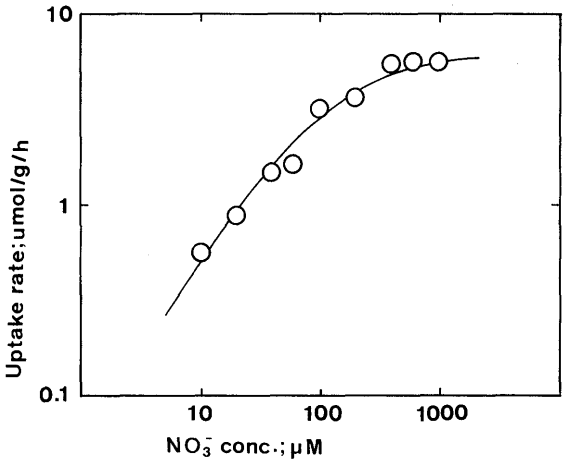

Fig. 1. Nitrate uptake rate in relation to the initial nitrate concentration in 15-day-old seedlings of a *japonica* cultivar, Nipponbare. Michaelis-Menten equation was estimated from the data.

Table 1. Michaelis-Menten kinetic parameters, apparent Km and apparent Vmax, for nitrate uptake by 15-day-old seedlings at 28℃ in 16 rice cultivars

(Average of three replications, m±S. D.)

Cultivar	Km (μM)	Vmax (μmol/g fw/h)
japonica		
Akage	97.7 ± 14.3	5.60 ± 1.35
Iwaga	75.7 ± 8.7	4.59 ± 0.39
Kinmaze	107.2 ± 17.3	5.76 ± 0.61
Koshihikari	81.9 ± 10.3	5.47 ± 0.41
Nipponbare	120.5 ± 5.0	7.10 ± 1.33
Norin 8	119.2 ± 7.8	7.17 ± 1.15
Tsutsu	101.3 ± 18.3	5.74 ± 0.65
Yamadanishiki	95.7 ± 11.1	5.90 ± 0.72
Mean	99.9 ± 16.0	5.92 ± 0.85
LSD (5%)	21.45	1.570
indica		
Daw Dam (Thailand)	82.2 ± 3.5	4.30 ± 0.26
f 145 (India)	124.3 ± 6.4	6.07 ± 0.69
IR 28 (Philippines)	130.3 ± 14.7	5.84 ± 1.26
Jamuna (India)	107.6 ± 11.5	4.81 ± 0.73
Leuang Tawng (Thailand)	118.7 ± 6.1	6.22 ± 1.11
Ma Sho (Myanmar)	90.0 ± 12.3	5.42 ± 0.67
Surjamkhi (India)	112.4 ± 20.6	6.45 ± 2.18
Wai Kyaku Nan Toku (China)	106.7 ± 22.6	5.25 ± 0.94
Mean	109.0 ± 16.4	5.55 ± 0.74
LSD (5%)	24.29	1.938

sentative Japanese modern cultivars, Nipponbare and Koshihikari. Koshihikari had lower apparent Km and apparent Vmax than Nipponbare.

Kinetic parameters of nitrate assimilation-related mutants

M605, which had been isolated as a chlorate-hypersensitive mutant, had remarkably (but not significantly, $0.1 > P > 0.05$) higher apparent Km than the original cultivar, Norin 8, while apparent Vmax of the mutant was almost the same level as that of the original cultivar (Table 2). In M605, nitrate uptake ability at the low nitrate concentration (below 100 μM) was lower than that of the original cultivar (Fig. 3). On the other hand, there were no clear differences in Km among the mutants M819, M1004 and NR827 and their original cultivars.

Discussion

In higher plants, nitrate uptake through the plasma membrane consists of two components, influx and efflux

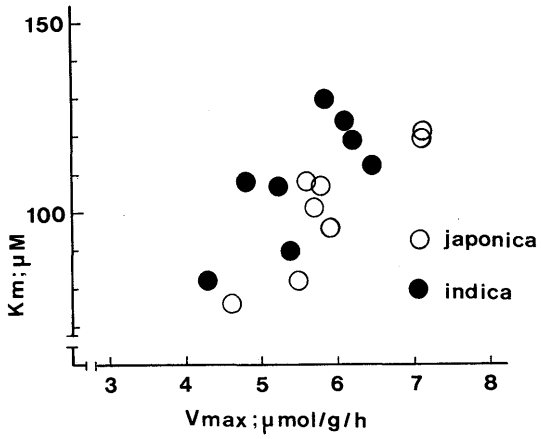

Fig. 2. Relationship between apparent Km and apparent Vmax for the nitrate uptake at 28 ℃ by 15-day-old seedlings in 16 rice cultivars (open and solid circles show *japonica* and *indica* cultivars, respectively).

Table 2. Kinetic parameters, apparent Km and apparent Vmax, for nitrate uptake by 15-day-old seedlings at 28℃ in nitrate assimilation-related mutants

(Average of three replications, m±S. D.)

Line	Km (μM)	Vmax (μmol/g fw/h)
M 605 (chlorate hypersensitive)	221.1 ± 63.8	6.99 ± 1.03
M 819 (nitrate reductase-deficient)	97.1 ± 14.2	6.36 ± 1.41
M 1004 (chlorate resistant)	113.7 ± 18.3	7.09 ± 0.35
Norin 8 (original cultivar of M lines)	119.2 ± 7.8	7.17 ± 1.15
NR 827 (low nitrate reductase activity)	140.8 ± 14.3	7.04 ± 0.87
Nipponbare (original cultivar of NR 827)	120.5 ± 5.0	7.16 ± 1.33

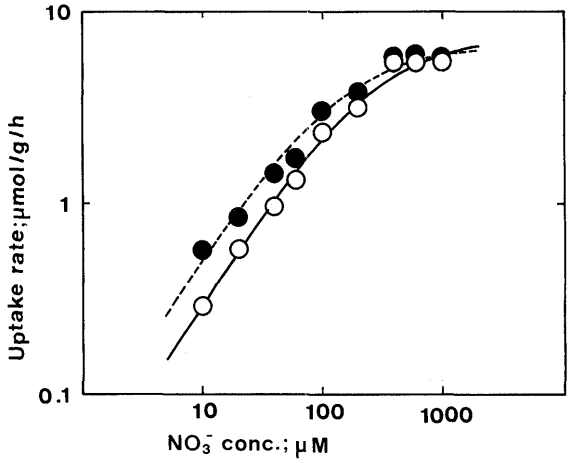

Fig. 3. Difference in nitrate uptake between a mutant line M 605 (open circles) and the original cultivar, Norin 8 (solid circles), as described by Michaelis-Menten kinetics.

(Deane-Drummond and Glass 1983). However, net uptake of nitrate can also be analyzed with Michaelis-Menten kinetics (Hasegawa 1992). This study was carried out based on the assumption that apparent Km and apparent Vmax calculated from the net uptake measurement during short period reflect the influx carrier ability.

In several plant species, it has been demonstrated that nitrate uptake by roots of young seedlings can be described by Michaelis-Menten kinetics at the concentrations below 1mM (Rao and Rains 1976, Doddema and Telkamp 1979, Konesky *et al.* 1989, Aguera *et al.* 1990, Laine *et al.* 1993). Furthermore, Pace and McClure (1986) reported the great variability in Km for nitrate uptake among 15 maize inbred lines. These results suggest that the inter- and intraspecific difference in nitrate uptake ability is due to the difference in the nitrate uptake gene(s), because Km represents the affinity of nitrate uptake carrier. However, as an experimental procedure for evaluating Km and Vmax for nitrate uptake has not been established at present, it is difficult to evaluate genetic nature of nitrate uptake from the kinetic parameters reported by different researchers.

Hasegawa (1992) examined the net nitrate uptake by young seedlings of 12 Japanese barley cultivars in 25 to 1,000 μM potassium nitrate solution supplemented with calcium sulfate and demonstrated that apparent Km and apparent Vmax at 25℃ varied from 50.9 to 111.7 μM and from 2.24 to 4.99 μmol/g fw/h, respectively. As the present experiment was carried out with almost the same method as described in the previous report, it is reasonable to compare the present data to those in barley reported by Hasegawa (1992). Apparent Km values of nitrate uptake in 16 rice cultivars ranged from 75.7 to 130.3 μmol/g fw/h, and were roughly coincident with the values obtained in Japanese barley cultivars. These results indicated that there may be no large difference in the genetic ability of nitrate uptake carrier between rice and barley. On the other hand, apparent Vmax in rice is higher than that in barley. Inter- and intra-

specific difference in apparent Vmax might be related to the difference in the modifying factors of nitrate uptake, such as energetics and growth regulation.

In the cultivars of *japonica* and *indica* which showed the similar level of apparent Km, *indica* cultivars tended to show lower apparent Vmax than *japonica* cultivars. Relatively lower values of apparent Vmax in *indica* cultivars might be explained as the difference in the energetics of nitrate uptake (plasma membrane H^+-ATPase activity, response to light intensity etc., Rao and Rains 1976 a, b, Sheahan *et al.* 1993) between *japonica* and *indica* rice plants. Cultivar difference in nitrate uptake in wheat was shown to be due the difference in the shoot relative growth rate (Rodger and Barneix 1988). For the improvement of the nitrate uptake in crop plants, it is also important to investigate the physiological and morphological traits modifying nitrate uptake ability.

Chlorate acts a nitrate analog and is catalized to toxic chlorite by nitrate reductase (NR) (Wray 1986). Chlorate resistance and hypersensitivity are useful selectable markers for NR-deficient and high NR activity mutant, respectively (Wang *et al.* 1986, Wray 1986). In rice, it is suggested that nitrate assimilatory ability differs between *japonica* and *indica* subspecies, because of the difference in chlorate sensitivity between the two subspecies. *indica* cultivars are more sensitive to chlorate than *japonica* (Morishima and Oka 1981, Ueno *et al.* 1990). In this experiment, there was no difference in Km values of nitrate uptake between *japonica* and *indica*. This fact indicates that the difference in nitrate assimilatory ability is not due to the nitrate uptake carrier but due to the nitrate reduction and the following biochemical steps. This is confirmed by another experiment (Hasegawa *et al.* 1992 b), which showed the elevated activities of both leaf nitrate reductase and leaf nitrite reductase in an *indica* cultivar, Leuang Tawng.

An interesting finding obtained in this study was that apparent Km of M605 was higher than that of the original cultivar without alteration of apparent Vmax. Increase in apparent Km value in M605 was due to the decrease in nitrate uptake rate at concentrations lower than 100 μM. M605 was isolated as a chlorate hypersensitive mutant (Hasegawa *et al.* 1992 b), but the relationship between the chlorate hypersensitivity and the decrease in nitrate uptake at the low nitrate concentration in M 605 is unclear. M605 can be a useful material for the study of nitrate assimilation in crop plants. Although low Km and high Vmax cultivars or lines were not found in this study, the finding of M605 suggests the possibility of altering nitrate uptake carrier by mutation in higher plants.

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