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A Chlorate-Hypersensitive, High Chlorate Uptake Mutant in Rice (*Oryza sativa* L.)

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Summary

A rice mutant hypersensitive to chlorate (an analog ion for nitrate), M605, was isolated from the mutant lines derived from a *japonica* cultivar, Norin 8. In this experiment, three biochemical steps of nitrate assimilation, i.e. nitrate uptake, nitrate reduction and nitrite reduction, were analyzed using M605, Norin 8 and a chlorate hypersensitive *indica* cultivar, Leuang Tawng. Uptake ability of monovalent anions including nitrate in M605 and Leuang Tawng was lower than that of Norin 8 except for chlorate uptake in M605. Chlorate uptake from a 200 μM potassium chlorate solution in M605 was 1.2 fold that in Norin 8. In both seedlings and roots of M605, activities of NADH-NR, NADPH-NR and NiR were similar to those of Norin 8. These results indicate that chlorate hypersensitivity in M605 may be due to the increased level of chlorate uptake. Genetic study showed that chlorate hypersensitivity in M605 was transmitted by polygenic factors. On the other hand, activities of leaf NRs and NiR of Leuang Tawng were twice to three times higher than those of Norin 8. There was no difference in the activities of root NRs and NiR between Leuang Tawng and Norin 8. It was suggested that the chlorate hypersensitivity in Leuang Tawng may be due to the higher activities of leaf NRs and NiR.

Key Words : *Oryza sativa* L., chlorate hypersensitivity, mutant, nitrate uptake, nitrate reductase.

Introduction

Improvement of the nitrate assimilatory ability in crop plants is one of the major objectives for breeding crops with efficient use of plant nutrients. For evaluating nitrate assimilation in higher plants, response to chlorate (a toxic analog ion for nitrate) at the seedling stage is a useful index. Chlorate resistance is a well-known selective marker for isolating mutants deficient in nitrate uptake and nitrate reductase (NR) in both cultured cell system and whole plant system (Wray 1986, Kleinhofs *et al.* 1989, Caboche and Rouze 1990). In rice, NR-deficient mutants (Hasegawa *et al.* 1991, 1992) and cell lines (Wakasa *et al.* 1984) were isolated as chlorate-resistant ones. On the other hand, chlorate-hypersensitive mutants isolated in *Arabidopsis thaliana* showed a higher NR activity or increased level of nitrate uptake (Wang *et al.* 1986, 1988).

Previously, Hasegawa *et al.* (1991) had reported that chlorate-hypersensitive mutants could possibly be included in the rice mutant lines (M lines) maintained at the Institute of Radiation Breeding, NIAR, Japan. In this paper, we report that one mutant hypersensitive to chlorate was isolated from the M lines. Nitrate assimilation of the mutant was analyzed and was compared with that of a chlorate hypersensitive *indica* cultivar.

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Materials and Methods

Plant Materials

Three rice mutant lines, M605, M1075 and M1101, and their original cultivar, Norin 8 (*japonica*), were used. These lines had been identified as possible chlorate-hypersensitive mutants in our previous study (Hasegawa *et al.* 1991). An *indica* cultivar, Leuang Tawng (a Thailand cultivar), was also used for the comparison of chlorate hypersensitivity.

Seedling growth in chlorate solution

The seeds were cultured in a 0 to 2 $\times$ 10^{-4}M potassium chlorate solution in a growth chamber at 25 ℃ under natural daylight supplemented by artificial light (16 h photoperiod). Twenty seeds were used in each treatment. The solution was exchanged every two days. On the 14th day after sowing, seedling height was measured as an index of chlorate sensitivity.

Measurement of monovalent anion uptake

Seedlings were grown in distilled water for 14 days and then were transferred into the solution containing 100 μM KNO_3 and 400 μM $CaSO_4$ for 24 h (pretreatment) at 25 ℃. On the 15 th day after sowing, five seedlings in each treatment were placed in a 50 ml solution of nitrate, nitrite, chlorate and chloride at 28 ℃, respectively. Each anion solution was applied as potassium salt at the concentration of 200 μM supplemented with 400 μM $CaSO_4$. After 2 h, the concentration of each anion was measured with an ion chromatograph (Dionex 2000i, U. S. A.) equipped with an anion selective column (IonPac

AS4A, Dionex, U. S. A.). Ion uptake was calculated based on the depletion from the solution. Each measurement was replicated three times.

Assays of nitrate reductases and nitrite reductase

For the analysis, seedlings were grown hydroponically in Kimura's B solution, which contained both nitrate and ammonium as nitrogen sources, in a greenhouse for 21 days under natural daylight and daylength conditions. Crude extracts from the leaves or the roots in each line were prepared. *In vitro* nicotinamide adenine dinucleotide, reduced form (NADH)-NR and nicotinamide adenine dinucleotide phosphate, reduced form (NADPH)-NR activities were assayed according to the method described by Hageman and Reed (1980). Nitrite reductase (NiR) activity was assayed according to the method of Ida and Mikami (1986). Each assay was replicated four times.

Inheritance of chlorate hypersensitivity in M 605

The F_2 seeds obtained from the reciprocal crosses of M605 and Norin 8 were cultured in a 10^{-4}M potassium chlorate solution in a growth chamber at 25 ℃. Seedling height was measured on the 14 th day after sowing. In each cross, 100 seeds were used.

Results

In all the cultivars and mutant lines used in this experiment, seedling height on the 14 th day after sowing decreased with the increase in the chlorate concentration (Fig. 1). In Norin 8, when the seedlings were cultured in 10^{-5}M potassium chlorate, seedling height was 76 % of that of the control, while 46 % of that of the control at 2×10^{-4}M chlorate. Among the M lines which had been identified as possible nitrate-hypersensitive mutants, seedling growth of M605 was most severely inhibited by chlorate. In particular, at a concentration of 10^{-5}M chlorate, seedling height of M605 was 43 % of that of Norin 8 (Fig. 1). On the other hand, the extent of seedling growth inhibition in M1075 and M1101 was intermediate between that of Norin 8 and M605. Therefore, only M605 was identified as a chlorate-hypersensitive line and was used for further experiments. As shown in Fig. 1, seedling growth of Leuang Tawng was more severely inhibited by chlorate than that of M605 except for the growth at 10^{-5}M chlorate.

When the F_2 seeds, which were obtained from the reciprocal crosses between M605 and Norin 8, were cultured in a 10^{-4}M potassium chlorate solution, the frequency distributions of the seedling height seemed to fit to a normal distribution as demonstrated in Fig. 2. The results suggested that the chlorate hypersensitivity in M605 was transmitted as a polygenic nuclear trait.

The monovalent anion uptake ability in M605 and Leuang Tawng was lower than that in Norin 8, except for the chlorate uptake in M605 (Table 1). Chlorate uptake ability in M605 increased to about 1.2-fold of that in Norin 8, while the uptake ability in Leuang Tawng was 64 % of that in Norin 8. Activities of NADH-NR,

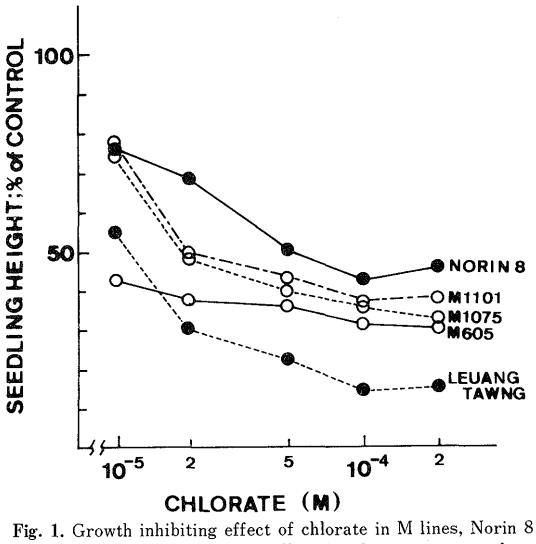

Fig. 1. Growth inhibiting effect of chlorate in M lines, Norin 8 and Leuang Tawng. Seedling height was measured on the 14 th day after sowing. Control means the seedling height of two cultivars and each mutant line grown distilled water for 14 days.

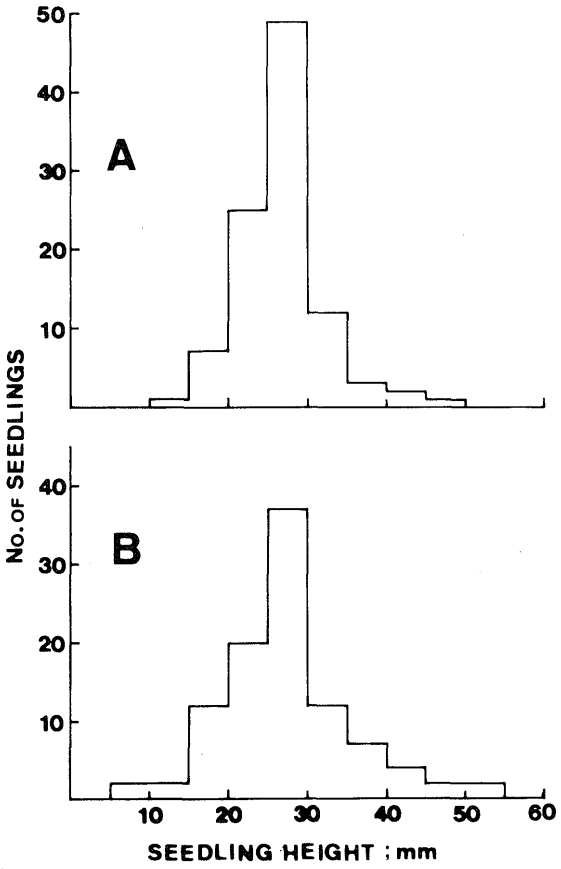

Fig. 2. Distribution of seedling height in the reciprocal crosses of M605 and Norin 8. Seedlings were cultured in 10^{-4}M potassium chlorate solution for 14 days.
A ; Norin 8 (♀) x M 605 (♂)
B ; M 605 (♀) x Norin 8 (♂)

Table 1. Uptake ability (μmol/g fresh weight/h) of four monovalent anions in Norin 8, M605 and Leuang Tawng (Average of three replications, m ± S. D.)

Line	Nitrate	Nitrite	Chlorate	Chloride
Norin 8	5.62 ± 0.10	5.09 ± 0.21	1.56 ± 0.30	8.30 ± 0.35
M605	4.91 ± 0.52	4.37 ± 0.10**	1.85 ± 0.55	7.01 ± 0.49*
Leuang Tawng	4.01 ± 0.04**	3.18 ± 0.26**	1.00 ± 0.10**	6.11 ± 0.51**

* and ** : Significantly different from Norin 8 at 5 and 1 % level, respectively.

Table 2. Activities of NADH-NR, NADPH-NR and NiR in Norin 8, M605 and Leuang Tawng. (Average of four replications, m ± S. D.)

	NADH-NR[a]		NADPH-NR[a]		NiR[b]	
Line	leaf	root	leaf	root	leaf	root
Norin 8	105.5 ± 12.8	24.8 ± 3.1	0.69 ± 0.36	1.75 ± 0.30	39.9 ± 7.7	52.2 ± 4.0
M605	119.0 ± 15.5	22.1 ± 3.7	0.61 ± 0.04	2.09 ± 1.16	36.3 ± 4.9	49.4 ± 4.3
Leuang Tawng	271.9 ± 16.8**	20.9 ± 3.9	2.09 ± 1.16**	1.76 ± 0.31	69.6 ± 5.1**	52.1 ± 1.6

[a] : nmol NO_2^- produced/g fresh weight/min
[b] : nmol NO_2^- reduced/g fresh weight/min
** : Significantly different from Norin 8 at 1 % level

NADPH-NR and NiR are shown in Table 2. In both leaves and roots of M605, activities of the three enzymes were almost similar to those of Norin 8. On the other hand, the activities of NADH-NR, NADPH-NR and NiR in the leaves of Leuang Tawng were 2.3, 3.4 and 1.9 times higher than those of Norin 8, respectively. However, no differences in the activities of root NRs and NiR were detected between Leuang Tawng and Norin 8.

Discussion

Wang *et al.* (1986, 1988) reported that several chlorate hypersensitive mutants of *Arabidopsis thaliana* were selected from an M_2 population and the mutants were divided into two groups. One mutant, C-4, showed a high leaf NR activity (1.25-fold of that of the wild type) and the chlorate hypersensitivity was inherited as a single recessive nuclear trait. Nitrate uptake and activities of NiR and glutamine synthetase in C-4 were normal (Wang *et al.* 1986). In another mutant, C-1, the chlorate hypersensitivity was due to the increase in uptake rates of both nitrate and chlorate, but the inheritance of the mutant trait was not ascertained (Wang *et al.* 1988).

In the current experiment, a chlorate hypersensitive rice mutant, M605, was isolated. There were no differences in the nitrate uptake and activities of NRs and NiR between M605 and the original cultivar, Norin 8. In M605, the chlorate uptake ability was higher than that of Norin 8, whereas the uptake ability of other monovalent anions was lower than that of Norin 8. Genetic study showed that chlorate hypersensitivity in M605 was controlled by polygenic factors. These results suggested that the M605 mutant was similar to the *Arabidopsis* C-1 mutant, although in the C-1 mutant the nitrate uptake rate was also high (Wang *et al.* 1988). In another experiment (Hasegawa and Ichii 1994), it was demonstrated that M605 showed a higher Km for the high affinity nitrate uptake system. However, at present, the relationship between chlorate hypersensitivity and higher Km for nitrate uptake in M605 is unclear.

On the other hand, the chlorate hypersensitivity in the *indica* cultivar Leuang Tawng seemed to be controlled by a different mechanism from that of M605. In the leaves of Leuang Tawng, the levels of NADH-NR, NADPH-NR and NiR were twice to three times higher than those of Norin 8. The uptake ability of four monovalent anions in Leuang Tawng was significantly lower than that in Norin 8. These results indicated that the chlorate hypersensitivity in Leuang Tawng may be due to the increase in the NR activity as demonstrated in the *Arabidopsis* C-4 mutant (Wang *et al.* 1986). Although no genetic analysis was carried out in Leuang Tawng in this study, it was reported that the inheritance of chlorate hypersensitivity in *indica* cultivars was complex (Sato 1991). It is well-documented that *indica* cultivars are more sensitive to chlorate than *japonica* cultivars (Morishima and Oka 1981, Ueno *et al.* 1990, Sato 1991). The present results showing that the activities of leaf NRs and NiR in Leuang Tawng were higher than those of Norin 8 suggest that the difference in the chlorate sensitivity between the two subspecies could be ascribed to the difference in the ability of leaf to assimilate nitrate.

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