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Genotypic Variations of Maize Seedlings in Utilizing Ammonium Nutrition

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ABSTRACT: In an effort to evaluate the ability of maize inbreds to utilize ammonium-nitrogen (NH_4^+-N), seedlings were grown in sand culture and supplemented with different amounts and forms of N. Seedlings were harvested at 28 days after planting and determined for gross differences in dry mass, activity of NH_4^+-assimilating enzymes, PEP carboxylase, and the ability to take up NH_4^+ ion. The results indicate that some inbreds retained the ability to effectively utilize NH_4^+, but they adapted various combinations of mechanisms to cope with NH_4^+ pressure and thereby allowed seedling growth and development in the presence of high NH_4^+ concentrations. Thus, plant breeders should be able to exploit this variability and to create hybrids with enhanced performance under predominantly NH_4^+-N conditions.

KEY WORDS: Ammonium, Ammonium assimilation, Maize, Nitrate, Nitrogen nutrition, *Zea mays L.*

INTRODUCTION

Nitrogen (N) is one of the major factors affecting production efficiency of crops and impacting ground water quality. Most of the soil N available for plant uptake is in the form of nitrate (NO_3^-) due to nitrification although ammonium (NH_4^+) is the most prevalent form of N fertilizer applied. Unlike NH_4^+ ion, NO_3^- is negatively charged so that it cannot be bound to the soil particles and thereby is subjected to leaching into ground water. As the concern over NO_3^- pollution increases, NH_4^+ nutrition will undoubtedly become more important for crop production especially when high efficient nitrification inhibitors are being developed as N management tools.

Both NH_4^+ and NO_3^- forms of N can be taken up by maize plants; however, metabolic efficiency of these two N forms is different. NH_4^+ is utilized more efficiently than NO_3^- for protein synthesis (Schrader *et al.*, 1972), and tissues accumulate a higher concentration of N from NH_4^+-N than from NO_3^--N (Dibb and Welch, 1976; Handa *et al.*, 1984). Furthermore, NO_3^- absorbed after the mid-silk stage may not be utilized for grain filling (Friedrich *et al.*, 1979; Friedrich and Schrader, 1979), and certain prolific genotypes are unable to take up NO_3^- during the period of kernel development (Pan *et al.*, 1984).

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Although NH_4^+ may be metabolically efficient, a high concentration of NH_4^+ as the only N source could create a "carbon stress" to restrict growth and development. This occurs because a small accumulation of NH_4^+ in maize tissues may be toxic (Matsumoto *et al.*, 1971; Anderson and Done, 1977); therefore, in response to a higher concentration of NH_4^+ in roots, a large proportion of the carbon skeletons, which are derived from the activity of photosynthetic enzymes such as phosphoenolpyruvate (PEP) carboxylase, may be shunted into an NH_4^+-assimilating pathway via glutamate synthase (GOGAT)/glutamine synthetase (GS) system, and glutamate dehydrogenase (GDH) to produce organic N compounds at the expense of other metabolic processes. When plants were grown under high NH_4^+-N conditions, high concentrations of asparagine and glutamine accumulated (Reisenauer, 1978; Tills and Alloway, 1981). These observations were consistent with reports that higher vegetative growth was obtained when maize plants were grown with mixtures of NO_3^--N and NH_4^+-N than with either NH_4^+-N or NO_3^--N alone (Schrader *et al.*, 1972; Handa *et al.*, 1984). Similar observations were reported with wheat (Cox and Reisenauer, 1973). Thus, the mixture of NO_3^- and NH_4^+ ions in plants may function as a "buffer" for optimizing nitrogen utilization.

In spite of the high efficiency of NH_4^+ nutrition for crop production, maize hybrids may not perform well under NH_4^+-N exclusively. This is because breeding programs and selection have been conducted under predominantly NO_3^--N environments that could result in eliminating the trait of high efficiency in NH_4^+ assimilation. Therefore, as a first step towards developing maize hybrids capable of performing under NH_4^+-N, domesticated germplasms were evaluated for the efficiency of NH_4^+ assimilation which includes activities of NH_4^+ assimilating enzymes, photosynthetic enzymes such as PEP carboxylase, and the ability of NH_4^+ uptake.

MATERIALS AND METHODS

Plant materials and growth conditions

In order to study genetic variability in NH_4^+ utilization, 11 inbreds, i.e., A661, H95, Va26, Hi31, PH9, CI66, ICAL210, A632, TA85-17, Pi77-4239, and Pi6875-370, which represent various genetic background and different degrees of sensitivity to NH_4^+ were selected for growth analysis under different forms of N. Seedlings (3 pot^{-1}) were grown in sand in a series of 17.5 x 12.8 cm (width x depth) plastic pots at 28℃ in a controlled climate room with a 16-h photoperiod at 10,000 Lux. One week after planting, each pot was flushed every day with 500 ml of modified Hoagland solution containing varying amounts and forms of N as indicated. The modified Hoagland solution consisted of 2.5 mM K_2SO_4, 4.0 mM $MgSO_4$, 1.0 mM KH_2PO_4, 1.1 mM $CaCl_2 \cdot 2H_2O$, 23 μM H_3BO_3, 46 μM $MnSO_4$, 15 μM $ZnSO_4$, 1.6 μM $CuSO_4 \cdot 5H_2O$, 0.8 μM Na_2MoO_4, and 100 μM Fe-EDTA (Handa *et al.*, 1984). The pH was adjusted to 6.5 with KOH. Seedlings were harvested two weeks after watering with the modified Hoagland solution, separated into roots and shoots, and oven-dried at 70℃ to determine dry weight. Each value represents the mean of four replications.

For studying the activities of PEP carboxylase and NH_4^+-assimilating enzymes, seedlings were grown with a similar protocol described above. Two weeks after planting, each pot was watered every other day with modified Hoagland solution containing 50 mM of N as $(NH_4)_2SO_4$ to maximize ammonium pressure. One week after watering with the

modified Hoagland solution, seedlings were harvested, and separated into roots and shoots. The harvested samples were divided into two lots: one lot was placed in an oven at 70℃ for dry weight determinations, root and shoot tissues in the second lot were washed, blotted, weighed, and frozen in liquid nitrogen before determining the activity of enzymes.

For studying the uptake of NH_4^+ ions, seedlings were grown with a similar protocol described above. Two weeks after planting, each pot was watered every other day with modified Hoagland solution containing zero N. One week later, seedlings were harvested for measuring the uptake of NH_4^+ ions.

GDH, GOGAT, GS extraction and assay

GDH, GOGAT and GS were extracted from maize roots according to the procedure described by Handa *et al.* (1985). One gram frozen root tissue was ground to a fine powder in liquid N with a mortar and pestle. The powdered sample was transferred into 5 ml of ice cold extraction buffer containing 100 mM imidazole-HCl, pH 7.5, and 10 mM 2-mercaptoethanol. The sample was homogenized for 30 seconds (Brinkman model PCU11), filtered through two layers of Miracloth, and then centrifuged at 18,800xg for 15 minutes at 4℃. The supernatant was then passed through a G-25 Sephadex column (1.5 x 15 cm) which was equilibrated in extraction buffer. Fractions containing the crude enzyme source were pooled and stored on ice until assayed.

GDH was assayed in the aminating direction by a modified method of Wallace (1973). The 1 ml assay mixture contained 100 μmol Tris-HCl, pH 8.0, 100 μmol $(NH_4)_2SO_4$, 10 μmol α-ketoglutarate, 0.1 μmol NADH, and 200 μl crude enzyme preparation. The assay, initiated by adding NADH, followed the oxidation of NADH in a 1 cm light path quartz cuvette at 340 nm. The assay was carried out at 25℃, and the blank contained no α-ketoglutarate.

GOGAT was assayed using a modified method of Dougall (1974). The 1 ml assay mixture contained 50 μmol HEPES, pH 7.5, 10 μmol glutamine, 10 μmol α-ketoglutarate, 0.1 μmol NADH, and 400 μl of the enzyme preparation. This assay was initiated by the addition of NADH, and followed spectrophotometrically at 340 nm for 5 min. The assay was done at 25℃, and blanks contained no α-ketoglutarate and glutamine.

A modification of the method of Kanamori and Matsumoto (1972) was used to assay GS activity. The 1 ml assay mixture contained 100 μmol imidizole-HCl, pH 7.5, 10 μmol 2-mercaptoethanol, 40 μmol $MgSO_4$, 10 μmol ATP, 50 μmol glutamate, and 10 μmol hydroxylamine (NH_2OH). After adding 200 μl of crude enzyme source, the reaction was incubated at 37℃ for 15 minutes. The reaction was terminated by adding 1 ml ferric chloride reagent (equal volumes 10% $FeCl_3 \cdot H_2O$ in 0.2 N HCl, 24% TCA, and 50% HCl), and centrifuged at 18,800xg for 10 minutes. The hydroxamate-derivative formed was determined at 540 nm at 25℃. The standard used was γ-glutamyl-hydroxamate, and the blanks contained no glutamate.

PEP carboxylase extraction and assay

One g of leaf tissue was ground to a fine powder in liquid nitrogen with a mortar and pestle. This sample powder was transferred into 5 ml of ice cold extraction buffer containing 100 mM HEPES, pH 7.4, 5 mM DTT, and 100 mg ml^{-1} insoluble PVP (Smith *et al.*, 1989).

The sample was then homogenized for 30 seconds and centrifuged at 26,000xg for 15 minutes at 4℃. After centrifugation, an aliquot of the supernatant was passed through a G-25 Sephadex column (1.5 x 15 cm) which was equilibrated in extraction buffer without PVP. The desalted sample containing the crude enzyme source was stored on ice until assayed. Since PEP carboxylase was found to be unstable, assays were routinely performed within 30 minutes after extraction.

PEP carboxylase was assayed using a modification of the method of Podestra and Andreo (1989). The 1 ml assay mixture contained 50 μmol HEPES-HCl, pH 7.0, 5 μmol $MgCl_2$, 5 μmol $NaHCO_3$, 0.16 μmol NADH, 1 μmol PEP, 4 units malate dehydrogenase, and 50 μl crude enzyme preparation containing PEP carboxylase. The reaction was initiated by adding the enzyme preparation, and was followed for 5 min in a 1 cm light path quartz cuvette spectrophotometrically at 340 nm. The assay was performed at 25℃, and PEP was omitted from the blank.

Determination of ammonium uptake

Harvested seedlings were washed with deionized water and immediately placed in ammonium free distilled water. The seedling was then transferred to 50 ml of an aerated NH_4^+ solution, pH 6.0 with $Ca(OH)_2$, as described by Topa and Jackson (1988). Incubations were performed on a laboratory bench at 24℃ ±2℃. Ammonium uptake (depletion from solution) was determined by the colorimetric indophenol reaction described by McCullough (1967). Reagent '1' (1L) contained 10 g phenol and 50 mg sodium nitroprusside, and reagent '2' (1L) contained 5 g sodium hydroxide, 53.7 g sodium phosphate dibasic, and 10 ml sodium hypochlorite (10-14%). After incubation at 37℃ for 35 minutes, the absorbance was determined at 625 nm. Ammonium sulfate was used as the standard.

Soluble protein determination

Protein in 200 μl of crude enzyme extract was precipitated with equal volumes of 20% TCA, placed at 4℃ overnight, and centrifuged at 26,000xg for 10 minutes. The supernatant was discarded and the pellet resuspended in 0.25N NaOH. The protein in each sample was subsequently quantified using the method of Bradford (1976).

RESULTS AND DISCUSSION

Effects of ammonium nutrition on dry matter accumulation

A previous study showed that the maximum level of NH_4^+-N maize seedlings could tolerate was about 10 mM (Xu *et al.*, 1992); therefore, maize inbreds were grown at concentrations higher than 10 mM to evaluate for their sensitivities of seedling growth to NH_4^+ nutrition. Since shoot (Table 1) and root (Table 2) tissues exhibited a similar growth pattern in responding to NH_4^+-N, the dry weight of these two tissues were combined in subsequent studies. As expected, seedling growth for most inbreds was retarded when NH_4^+ concentrations were higher than 10 mM (Tables 1 to 3).This observation is expected because of breeding programs and selection under predominantly NO_3^- environments which might inadvertently eliminate the trait for NH_4^+ utilization. Unlike NO_3^-, NH_4^+ is relatively toxic at low concentrations *in vivo* (Matsumoto *et al.*, 1971; Anderson and Done, 1977) and must be assimilated immediately in the root by the reductive amination of an available carboxylic

Table 1. Effects of ammonium concentrations on shoot dry matter accumulation in maize inbreds.

Inbred	NH_4^+ concentration			
	10 mM	20 mM	30 mM	40 mM
	Dry weight (g/seedling)			
A661	1.50	1.13	0.90	0.56
H95	1.36	1.06	0.80	0.66
Va26	0.85	0.70	0.52	0.51
Hi31	0.72	0.53	0.44	0.32
PH9	0.73	0.57	0.53	0.45
CI66	0.85	0.66	0.53	0.41
ICAL210	0.60	0.62	0.48	0.28
A632	0.61	0.45	0.43	0.24
TA85-17	0.49	0.46	0.45	0.27
Pi77-4239	0.62	0.51	0.54	0.38
Pi6875-370	1.02	0.92	0.92	0.42

Seedlings (3 pot^{-1}) were grown in sand in a series of 17.5 x 12.8 cm plastic pots at 28℃ in a controlled climate room with a 16-h photoperiod. One week after planting, each pot was flushed every day with 500 ml of modified Hoagland solution containing 10 to 40 mM N as $(NH_4)_2SO_4$. Seedlings were harvested two weeks after watering with the modified Hoagland solution. Conc. $LSD_{0.05} = 0.07$; Inbred $LSD_{0.05} = 0.10$.

Table 2. Effects of ammonium concentrations on root dry matter accumulation in maize inbreds.

Inbred	NH_4^+ concentration			
	10 mM	20 mM	30 mM	40 mM
	Dry weight (g/seedling)			
A661	0.51	0.45	0.37	0.22
H95	0.44	0.42	0.37	0.35
Va26	0.32	0.26	0.23	0.22
Hi31	0.27	0.28	0.24	0.17
PH9	0.24	0.20	0.20	0.16
CI66	0.32	0.30	0.22	0.20
ICAL210	0.20	0.22	0.18	0.10
A632	0.29	0.27	0.26	0.12
TA85-17	0.20	0.16	0.17	0.13
Pi77-4239	0.26	0.25	0.27	0.18
Pi6875-370	0.35	0.34	0.30	0.20

Seedlings (3 pot^{-1}) were grown in sand in a series of 17.5 x 12.8 cm plastic pots at 28℃ in a controlled climate room with a 16-h photoperiod. One week after planting, each pot was flushed every day with 500 ml of modified Hoagland solution containing 10 to 40 mM N as $(NH_4)_2SO_4$. Seedlings were harvested two weeks after watering with the modified Hoagland solution. Conc. $LSD_{0.05} = 0.05$; Inbred $LSD_{0.05} = 0.06$.

Table 3. Effects of ammonium concentrations on total seedling dry matter accumulation in maize inbreds.

	NH_4^+ concentration			
Inbred	10 mM	20 mM	30 mM	40 mM
	Dry weight (g/seedling)			
A661	2.01	1.58	1.27	0.78
H95	1.80	1.48	1.17	1.01
Va26	1.17	0.96	0.75	0.73
Hi31	0.99	0.81	0.68	0.49
PH9	0.97	0.77	0.73	0.61
CI66	1.15	0.96	0.75	0.62
ICAL210	0.80	0.84	0.66	0.38
A632	0.90	0.72	0.69	0.36
TA85-17	0.69	0.62	0.62	0.40
Pi77-4239	0.88	0.76	0.81	0.56
Pi6875-370	1.37	1.26	1.22	0.62

Seedlings (3 pot^{-1}) were grown in sand in a series of 17.5 x 12.8 cm plastic pots at 28 ℃ in a controlled climate room with a 16-h photoperiod. One week after planting, each pot was flushed every day with 500 ml of modied Hoagland solution containing 10 to 40 mM N as $(NH_4)_2SO_4$. Seedlings were harvested two weeks after watering with the modified Hoagland solution. Conc. $LSD_{0.05}$ = 0.10; Inbred $LSD_{0.05}$ = 0.14.

Table 4. The effect of N forms (30 mM) on total seedling dry matter accumulation in maize inbreds.

	NH_4 (mM)/NO_3 (mM)			
Inbred	30/0	20/10	10/20	0/30
	Dry weight (g/seedling)			
A661	1.28	2.23	2.06	1.94
H95	1.39	1.66	2.22	1.94
Va26	0.76	1.07	1.23	1.21
Hi31	0.62	1.17	1.14	1.49
PH9	0.73	0.86	1.06	1.09
CI66	0.99	1.42	1.79	1.75
ICAL210	0.63	0.95	1.20	1.21
A632	0.92	0.98	1.29	1.20
TA85-17	0.39	0.65	0.83	0.81
Pi77-4239	0.60	1.07	1.15	1.63
Pi6875-370	1.46	1.75	2.15	1.70

Seedlings (3 pot^{-1}) were grown in sand in a series of 17.5 x 12.8 cm plastic pots at 28 ℃ in a controlled climate room with a 16-h photoperiod. One week after planting, each pot was flushed every day with 500 ml of modified Hoagland solution containing 30 mM N as $(NH_4)_2SO_4$, KNO_3, or their mixtures in a ratio of 2:1, or 1:2. Seedlings were harvested two weeks after watering with the modified Hoagland solution. Conc. $LSD_{0.05}$ = 0.11; Inbred $LSD_{0.05}$ = 0.28.

acids to form non-toxic amino acids prior to translocation (Raven and Smith, 1976; Ivanko and Ingversen, 1971). The assimilation of NH_4^+ and subsequent organic N interconversions require organic acids which are derived from sucrose. Thus, a high concentration of NH_4^+ in roots could create a physiological pressure enhancing sucrose translocation from leaves to roots for its assimilation at the expense of other metabolic processes. This could explain a low vegetation weight observed for seedlings grown with predominantly NH_4^+-N when the total N level was high (Table 1). Therefore, a mixture of NH_4^+ and NO_3^- should provide a better condition for seedling growth because NH_4^+ may enhance the movement of sucrose from leaves to relief "feedback inhibition" of photosynthesis and nitrate functions as a nitrogen reserve (which may be accumulated without utilization). As shown in Table 4, all inbreds tested preferred mixtures of NH_4^+ and NO_3^- for growth. These observations are consistent with the notion that a mixture of N forms may function as a "buffer" for optimizing nitrogen utilization.

Although most maize inbreds evaluated preferred mixtures of NH_4^+ and NO_3^-, certain inbreds such as A661 could tolerate a high proportion of N in NH_4^+ form for growth. These observations suggest that these NH_4^+-tolerant inbreds might adapt a specific biochemical mechanism for a more effective utilization of NH_4^+-N.

Biochemical traits of ammonium utilization

There are three enzymes implicated in NH_4^+ assimilation in higher plants; namely, GOGAT, GS, and GDH. It is now widely accepted that the main pathway for assimilation is via the GOGAT/GS cycle first proposed by Lea and Miflin (1974). However, the assimilatory role of GDH could not be ruled out. The apparent Km of GDH for NH_4^+ inplants ranges from 10 to 50 mM (Miflin and Lea, 1977), indicating its low affinity for the substrate and apparent inability to operate under conditions where NH_4^+ concentrations are low (i.e. normal cellular NH_4^+ levels). However, the validity of this argument may be questionable because the NH_4^+ concentration in the rhizosphere may become high enough to allow GDH in roots to function in an assimilatory pathway. In fact, a great deal of evidence supports the assimilatory role of GDH in response to elevated levels of NH_4^+ since its activity has repeatedly been shown to increase in roots of corn, tomato, rice, and pea (Loyola-Vargus and DeJiminez, 1984; Handa *et al.*, 1985; Magalhaes and Huber, 1991), as well as in suspension cultures of *Lemna minor* and *Ipomoea* (Rhodes *et al.*, 1976; Zink, 1989) as a direct result of excessive NH_4^+ concentrations. Since NH_4^+ assimilation and carbon metabolism are closely linked (Givan, 1979), seedlings may encounter periods of carbon depletion during active NH_4^+ assimilation unless additional carbon substrates could be available for growth and metabolic processes. This may be controlled, in part, by inbred ability to differentially increase activities of enzymes involved in carbon assimilation in response to NH_4^+ pressure. Because NH_4^+ assimilation involves organic carbon skeletons (derived from PEP and RuBP carboxylase activities), assimilating enzymes (GDH, GS, and GOGAT), and NH_4^+ uptake, efforts were made to determine which factors are responsible for the ability or inability of maize inbreds to utilize NH_4^+ nutrition. As shown in Table 5, the correlation coefficient between dry weight accumulation and the activity of GS, GOGAT , GDH, or PEP carboxylase among the 11 inbreds studied was very low. Likewise, there was no correlation between dry weight and NH_4^+ uptake (Table 6), soluble proteins in roots or

Table 5. Relationship between seedling dry weight and enzyme activity of NH_4^+ assimilation and PEP carboxylase in maize inbreds.

Genotype	Dry weight	GS	GOGAT	GDH	PEPcase
	g/seedling	---------- Activity (unit/mg protein/min) -------			
A661	0.51	0.83	0.018	0.036	0.35
H95	0.52	1.30	0.071	0.021	0.39
Va26	0.84	0.67	0.018	0.015	0.29
Hi31	0.37	0.33	0.051	0.026	0.64
PH9	0.51	0.52	0.021	0.019	0.65
CI66	0.64	0.83	0.073	0.032	0.55
ICAL210	0.30	0.44	0.050	0.031	0.49
A632	0.43	0.97	0.049	0.034	0.22
TA85-17	0.30	0.67	0.009	0.028	0.51
Pi77-4239	0.34	0.24	0.040	0.023	----
Pi685-370	0.66	0.93	0.038	0.021	0.19

Unit for GS is μmoles γ-glutamyl-hydroxamate formed; Unit for GOGAT, GDH, and PEPcase is μmoles NADH oxidized. Correlation coefficients (Υ^2) between dry weight and enzymatic activity are: 0.09 for GS, 0.004 for GOGAT, 0.016 for GDH, and 0.24 for PEP carboxylase.

Table 6. Relationship between NH_4^+ uptake activity and seedling dry weight of maize inbreds.

Genotype	Dry weight	Uptake
	g/seedling	μmol/g fresh weight/h
A661	0.51	4.25
H95	0.52	1.45
Va26	0.84	2.25
Hi31	0.37	2.90
PH9	0.51	2.67
CI66	0.64	1.43
ICAL210	0.30	0.31
A632	0.43	1.26
TA85-17	0.30	1.56
Pi77-4239	0.34	1.64
Pi685-370	0.66	2.30

$\Upsilon^2 = 0.05$

soluble proteins in shoots (Table 7). From these data, it is clear that no one factor is entirely responsible for enhanced seedling growth with high NH_4^+ levels and that a more complex interaction between enzymes exists. This study indicates that certain inbred lines retain the ability to more effectively utilize ammonium, but adapt various combinations of mechanisms to cope with NH_4^+ pressure and thereby allow seedling growth and development in the presence of NH_4^+ in high concentrations. Even though NH_4^+ assimilation is not a simply controlled process, there appears to be much genetic variation among maize inbred lines in their ability to efficiently utilize NH_4^+. Plant breeders should be able to exploit this variability and create hybrids with enhanced performance under predominantly NH_4-N conditions.

Table 7. Relationship between seedling dry weight and soluble proteins in the root and shoot of maize inbreds.

Genotype	Dry weight	root	Shoot
	g/seedling	mg soluble proteins/g	fresh weight
A661	0.51	1.35	8.85
H95	0.52	0.96	8.97
Va26	0.84	1.54	4.75
Hi31	0.37	0.91	6.77
PH9	0.51	1.16	12.87
CI66	0.64	1.11	8.58
ICAL210	0.30	0.31	8.46
A632	0.43	1.85	8.49
TA85-17	0.30	1.56	9.91
Pi77-4239	0.34	1.27	----
Pi685-370	0.66	1.42	9.37

Correlation coefficients (Υ^2) between dry weight and root soluble proteins and shoot soluble proteins are 0.04 and 0.06, respectively.

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玉米利用銨態氮肥的遺傳差異性

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摘　　要

為了評估玉米品種利用銨態氮肥的能力，首先將幼苗生長於沙盆中，並施加不同形式及不等量之氮源。播種 28 天後將幼苗收穫並分別測定其乾重量，銨離子同化酵素和 PEP carboxylase 的活性，以及根部吸收銨離子的能力。所得結果顯示有幾個品種頗具利用銨離子的能力，且可以在高濃度的銨離子情況下生長。由於這幾個品種似乎各自採取不同的機制，因此育種專家應可利用這些品種間的差異性，而交配出具有高效率利用銨離子的雜交種。

關鍵詞：銨離子、銨離子同化作用、玉米、硝酸根離子、氮肥、*Zea mays* L.。

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