Difference in Tolerance to Potassium Deficiency between Two Maize Inbred Lines

Cao Minjian, Yu Haiqiu, Yan Hongkui and Jiang Chunji

(College of Agronomy, Shenyang Agricultural University, Shenyang, China 110161)

Abstract: Northeast China produces an abundance of maize (Zea mays L.), and improvement of maize yield at this region correlates closely with ensure food supply safely of China. In recent years, deficiency or relative deficiency of potassium (K) in the soil is an important limitation to maize production. Maize inbred lines tolerant to K deficiency (T) and sensitive to K deficiency (C) were hydroponically grown in 1/2 Hoagland solution to study the possible mechanism of maize tolerance to potassium deficiency from physiological point of view. With the reduction of K concentration, DW of the plant became apparently greater in T than in C, the symptom of potassium deficiency advanced in C. Under K deficiency, the taproot elongation increased and root top ratio decreased to a greater degree in C than in T. C had fewer lateral roots. T had a stronger K uptake ability than C, and the difference was more obvious in culture solution with a lower K concentrations of culture solution. K dependent H exchange activity, K uptake, Maize, Tolerance to K deficiency.

Key words: K/H exchange activity, K uptake, Maize, Tolerance to K deficiency.

Currently, it is estimated that only 10.1% of farmland in the world is stress-free soil suitable for the growth of crops (Jensen and Pettersson, 1980). Application of fertilizer is not a practical solution to this problem, because of limited material resources, the limited availability, high cost, low input of farmers, and environmental pollution resulting from excessive use of fertilizers in developed countries. Many scientists suggested that an alternative or complementary approach to solve the problem of nutrient deficiency in soil is the development of crops with greater adaptation to nutrient stress (Pettersson, 1978; Perby and Jensen, 1983; Lynch, 1998). Undoubtedly, it is important to reduce production costs, improve economic efficiency and protect the environment.

Northeast China is a major maize production area. The region is improtant for both cultivated area and output of the crop. In recent years, because of the deficiency or relative deficiency of potassium in the soil, improvement in maize yield has been constrained. The artificial reasons for potassium deficiency in soil are as follow: First, as the progress in cultural technology and the development of high yield hybrid, large amount of nutrient elements were absorbed by crop. Adding chemical fertilizers of nitrogen (N) and phosphorus (P) can compensate the deficiency of N and P in soil. However, potassium fertilizers have scarcely been applied to soil: K can be supplied slightly from organic fertilizer, but the majority of the farmland was not supplied with organic fertilizer. Therefore, the amount of potassium in the soil removed is more than that introduced. Secondly, large amounts of N and P in soil are not utilized by the crop under high yielding conditions, which breaks the balance of nutrition in the soil. Hence the amount of potassium in the soil could not meet the needs of corn growth. For these reasons a certain amount of potassium should be added to the soil. However, since China is poor in potassium mineral resources and is dependent on import for most of its potash fertilizer supply, the element is not supplied adequately.

We have obtained hundreds of specimens of maize-inbred lines and varieties since the collection was started in 1988. We planted them in potassium-deficient soils to select the potassium stress-tolerant inbred lines. Then we developed a number of strains that are tolerant to potassium deficiency through crossing and selection. When grown in potassium-deficient soils, these strains demonstrated an immense superiority, but their tolerance mechanism was not clear.

The uptake, transport and use efficiency of potassium has been studied in barley (Glass and Perley, 1980; Pettersson and Jensen, 1983), wheat (Siddiqi, 1987; Woodend et al., 1987; Agata et al., 2001) and tomato (Epstein, 1978; Makmuru et al., 1978; Figdor et al., 1989; Chen and Warren, 2000), but not in maize. Here, we studied the mechanism of tolerance of maize to potassium deficiency from physiological perspective through the difference between the two contrasting maize inbred lines under potassium deficiency.
Table 1. DW of T and C in the solution with different K’ concentrations.

| Item                        | K’ concentration (mM) |
|-----------------------------|-----------------------|
|                             | 0         | 0.625     | 1.25      | 2.5       |
| DW of shoot for T (g)       | 3.9       | 12.0      | 13.5      | 14.5      |
| DW of shoot for C (g)       | 1.3       | 6.7       | 10.9      | 13.6      |
| DW of root for T (g)        | 2.3       | 5.7       | 6.2       | 6.5       |
| DW of root for C (g)        | 0.9       | 3.9       | 5.3       | 6.6       |
| Shoot/Root ratio for T      | 1.69      | 2.11      | 2.17      | 2.22      |
| Shoot/Root ratio for C      | 1.48      | 1.74      | 2.04      | 2.06      |
| T/C ratio for shoot         | 3.03      | 1.80      | 1.23      | 1.07      |
| T/C ratio for root          | 2.85      | 1.40      | 1.16      | 0.99      |

Shoot LSD₀.₀⁵=1.07, Root LSD₀.₀⁵=0.215.

Materials and Methods

Maize inbred lines tolerant to K’ deficiency (T) and sensitive to K’ deficiency (C) were hydroponically grown in 1/2 Hoagland solution, in which Na’ was replaced by K’ because maize responds little to Na’. Different levels of K’ were supplied as KCl. Our group developed line T. Line C was a parent of hybrid Shendan 8 with high drought resistance, which is one of the most widely planted hybrids in Liaoning province of China.

The seeds were sterilized with 10% sodium hypochlorite (NaClO) for 20 min., washed with water and soaked in deionized water for 4 h. Then, the seeds were germinated by wet towel-roll method under 27 °C for 2 d. The germinated seeds were placed in reticulate-bottomed plastic containers touching water with ion in a pot for 1 week under room temperature.

The culture solution was Hoagland nutrition, with NaNO₃ and NaH₂PO₄ replaced by KNO₃ and KH₂PO₄, respectively. For seedling culture, the modified Hoagland nutrition was diluted to 1/2 of the original concentration. It contained (in μM): 2500 NaNO₃, 2500 Ca(NO₃)₂, 1000 MgSO₄, 5000 NaH₂PO₄, 50 EDTA-Fe (III)Na, 115 H₃BO₃, 22.5 MnCl₂, 0.75 CuSO₄, 0.75 ZnSO₄, and 0.182 (NH₄)₆MoO₂₄. Four levels of K’ (0, 0.625mM, 1.25mM and 2.5 mM) were supplied as KCl, with 2.5 mM as the standard.

Maize seedlings were transplanted to pots (four seedling per pot) containing 3L of the culture solution. Each treatment had three replications. The solution was renewed every 3 d. Air was pumped into the culture solution to provide oxygen to the roots. The seedlings were cultured in a naturally illuminated greenhouse for four weeks and then were examined for the number of leaves and the symptom of potassium deficiency. The completely dead leaf was regarded as the dead leaf, and that which showed a symptom of potassium deficiency and yet stayed partially green was designated as the injured leaf.

Adventitious root was included in the taproot, and the branch root growing out of the taproot was defined as the lateral root. The number of lateral roots per centimeter of taproot was defined as the density of lateral roots. Afterwards, the top and the roots of the plant were separated, washed, desiccated, weighted, crushed, decomposed by the wet-way method and the K’ content was determined by the flame photometer method (Center agricultural experimental station of Hokkaido, 1981).

Maize seedlings were cultured in the culture solution for 12 d in an incubator kept at 27/20°C(day/night). The solution was renewed once every 3 d. Then the seedlings were subjected to potassium starvation treatment in the culture solution without K’ for 3 d. The starvation-treated root segments (without lateral roots), 5cm from the root tip, with a fresh weight of 0.981〜1.023 g for each treatment were collected. The potassium-absorbing capacity of the root was measured with a K’ electrode in 200ml solutions (containing 0.5 mM Ca²⁺) with K’ at three concentrations 0.15, 0.10 and 0.05 mM, 3 replications with pH adjusted to 6.0 for 90 min mixing with magnetic bar. Measurements were recorded with an automatic recorder.

The same starvation treated roots were collected to measure the amount of K’/H’ exchange under solutions (containing 0.5 mM Ca²⁺) of 0, 0.125, 0.25, 0.5, 1.0 mM K’, 200 mL each, 3 replications with pH adjusted to 6.0 for 90 min following mixing with
magnetic bar. The same method as above mentioned. Fresh weight of each measured sample was between 0.962~1.027 g.

Results and Discussion

1. Difference in dry weight

Table 1 shows the dry weights (DW) of the shoot and the root of plant of the two inbred lines. With the reduction of K’ concentration in the culture solution, the DW of the shoot and root decreased was lighter in T than in C. For example, when the K’ concentration in the solution dropped from 2.5 to 0 mM, the shoot DW of the shoot for T fell from 14.5 g to 3.9 g, and that of C from 13.6 g to 1.3 g, and the relative weight fell from 100% to 26.7% and to 8.8%, respectively. The T/C ratio of shoot DW rose from 1.07 to 3.03. This trend of change in the root DW was similar to that for the shoot DW. With the reduction of K’ concentration, the top/root ratio of DW in the two inbred lines sank also, but to a lesser extent in T than in C.

2. Difference in symptom

Fig. 1 shows the symptom of potassium deficiency in the two inbred lines under different K’ concentrations. Normally, T has 19 leaves, while C has 21, suggesting the existence of a genetic difference in the number of leaves. In this experiment, upon sampling, T had 7 leaves in all treatments, while C had 9 leaves except in the no-K’ treatment (8 leaves). The one leaf shortage in the no-K’ treatment was caused by growth retardation due to lack of potassium. In C, all 9 leaves were green in the 2.5 mM K’ treatment and dead leaves and injured leaves occurred below that concentration and the symptom being advanced as the potassium concentration was further reduced. On the contrary, in T, symptoms of potassium deficiency appeared only in the no-K’ treatment.

3. Root development

Fig. 2 shows the pattern of root development. With the reduction of potassium concentration, the number of taproots decreased. There was no significant difference between T and C. The average length of taproots increased in both inbred lines, but the degree of increase was greater in C than in T. The density of lateral roots decreased in both inbred lines, but the drop was greater in T than in C. Thus there were significant differences between the two inbred lines. Under nutrient deficiency, crops tend to increase their roots and reduce their shoot/root ratio (Yan et al., 1995). In these two inbred lines, C was superior to T, but the former had fewer lateral roots than the latter. Root growth consumes photosynthesis and energy. In terms of root structure, C was inferior to T.

4. K’ contents of plant

Table 2 shows the K’ contents of the shoot and the root in the two inbred lines. By lowering the K’ concentration in the culture solution, the K’ contents in the shoot decreased from 4.36% to 0.93% in T and from 4.21% to 0.62% in C, while the ratio between K’

| Item                        | K’ concentration (mM) |
|-----------------------------|-----------------------|
| K’ contents in shoot of T(%)| 0.93  2.02  4.01  4.36 |
| K’ contents in shoot of C (%)| 0.62  1.53  3.61  4.21 |
| T/C ratio for shoot         | 1.50  1.32  1.11  1.04 |
| K’ contents in root of T (%)| 1.21  3.11  4.34  4.21 |
| K’ contents in root of C (%)| 0.68  2.04  3.72  4.41 |
| T/C ratio for root          | 1.78  1.52  1.17  0.95 |

LSD<sub><sub>0.05</sub></sub>=0.271.
contents of the shoot rose from 1.04 to 1.50. The K⁺ content of the root decreased from 4.21% to 1.21% in T and from 4.41% to 0.68% in C, while the ratio between K⁺ contents rose from 0.95 to 1.78. Hence, it was evident that under a high K⁺ concentration (2.5 mM), the two inbred lines had an equal amount of K⁺. With the reduction of K⁺ concentration, K⁺ level was apparently higher in T than in C. This change was especially significant in the roots.

5. K⁺ uptake by the roots

Fig. 3 shows the change of K⁺ uptake by the roots with time. When K⁺ concentration of the culture solution was 0.15 mM, the difference in K⁺ uptake by the roots between the two inbred lines was small. Within 90 minutes, K⁺ uptake per gram of fresh weight of roots was 6.63 µmol in T and 5.01 µmol in C, the T/C ratio was 1.32. When the K⁺ concentration was reduced to 0.10 mM, the ratio rose. Within 90 minutes, the K⁺ uptake was 5.32 µmol in T, but only 2.95 µmol in C, giving a ratio of 1.81. When the K⁺ concentration was lowered to 0.05 mM, the T/C ratio became even greater. Within 90 minutes, it increased to 2.32. These findings indicated that T had a stronger K⁺-absorbing capacity than C, and the difference became greater as the K⁺ concentration in the culture solution was lowered.

6. pH change in culture solution

Figure 4 shows the change of K⁺-dependent H⁺ extrusion from the roots with time. In the inbred line C when K⁺ concentration of culture solution was 1.0 mM, the pH of the solution decreased from 6.0 to 4.34 within 90 minutes. In T, at the same concentration and within the same duration, pH lowered from 6.0 to 3.19, and under 0.25 mM K⁺, from 6.0 to 4.43. This downward trend was appreciable even under a still lower K⁺ concentration (0.125 mM).

It is generally believed that potassium intake by the roots depends on H⁺-ATPase and K⁺ transporter in cell membrane. In other words, K⁺ is taken into the cell by the K⁺ transporter through the formation of H⁺ electrochemical potential (pH gradient and
membrane potential difference). There are two kinds of K' transporters: one is the H'/K' symport that has a high affinity for K', the other is the K' channel that has a low affinity for K' (Romheld et al., 1984; Rubnstein, 1992; Walter and Julian, 1994; Donald, 1995). For example, Ullrich and Jnovacky (1991) indicated that during a high K' absorption process, the accompanied depolarization of membrane potential by the action of H'-ATPase released H' that caused the pH to fall. In this study, using K'/H' exchange activity as an index, we measured the degree of pH drop induced by root-released H' in the solutions with different K' concentrations. The results indicated that T could release H' at low K' concentrations. The present findings indicated that the inbred line T had a stronger potassium-absorbing capacity. Hence, the H' extrusion rate could be a good measure for the tolerance to potassium deficiency and could be useful for quantitative trait loci analysis.

References
Agata, R., Mario, R. and Linda, M. 2001. Enhanced osmotic tolerance of a wheat mutant selected for potassium accumulation. Plant Sci. 160 : 441-448.
Jensen, P. and Pettersson, S. 1980. Varietal variation in uptake and utilization of potassium (rubidium) in high-salt seedlings of barley. Physiol. Plant. 48 : 411-415.
Lynch, J.P. 1998. The role of nutrient-efficient crops in modern agriculture. J. Corp Prod. 1 : 241-264.
Romheld, V., Muller, C. and Marschner, H. 1984. Localization and capacity of proton pumps in roots of intact sunflower plants. Physiol. Plant. 76 : 603-606.
Rubnstein, B., Sten, A.I., Jonathan, D.C. and Chalmers, J.D. 1994. The role of nutrient-efficient crops in modern agriculture. J. Corp Prod. 1 : 241-264.
Rubnstein, B., Sten, A.I., Jonathan, D.C. and Chalmers, J.D. 1994. The role of nutrient-efficient crops in modern agriculture. J. Corp Prod. 1 : 241-264.

Center Agricultural Experimental Station of Hokkaido. 1981. Standard of Diagnosis for Soil and Plant Nutr. 112-113

Chen, J.J. and Warren, H. G. 2000. Morphological and physiological characteristics of tomato roots associated with potassium-acquisition efficiency. Sci. Hortic. 83 : 213-225.

Donald, P. 1995. Characterization of the red beet plasma membrane H'-ATPase reconstituted in a planar bilayer system. Ibid. 108 : 393-398.

Epstein, E. 1978. An inborn error of potassium metabolism in tomato, Lycopersicon esculentum. Plant Physiol. 62 : 582-585.

Figgude, S., Gerloff, G.C. and Gabelman, W.H. 1989. The effect of increasing sodium chloride level on potassium utilization efficiency of tomato grown under low potassium stress. Plant Soil 119 : 295-304.

Glass, A.D.M. and Perley, J.E. 1980. Parietal difference in potassium uptake by barley. Plant Physiol. 65 : 160-164.