



EFFECT OF LOW POTASSIUM STRESS ON ROOT MORPHOLOGICAL CHARACTERISTICS AND POTASSIUM ACCUMULATION AT SEEDLING STAGE IN MAIZE [*Zea mays* L.]

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ABSTRACT

In present study, effect of various potassium (K) treatments on morphological characteristics of root, dry matter weight and K accumulation were evaluated in fifteen representative maize [*Zea mays* L.] inbred lines. The performance of root morphological traits, dry matter weight and K accumulation amount (KAA) were significantly different among various genotypes, K levels under low K stress. In addition, the interactions of genotype with K level were also significant except for total root length and root to shoot ratio. The total root length of LD9-1, ZD-2, 91-2, 099 and 90-21-3, were increased under low K treatment, while the root surface area, volume and average diameter were slightly decreased compared to other inbred lines. In addition, root dry weight, shoot dry weight and root to shoot ratio were not slightly affected by low K stress. On the other hand, ZD-1, D937, 8112, 835 were highly sensitive to low K stress. It is worthy note that the KAA of root, shoot and plant in LD9-1, ZD-2, 91-2, 099 and 90-21-3 were significantly higher than in other inbred lines under low K stress. The results showed that ZD-2, 91-2, 099 and 90-21-3 were tolerant to low K stress, while ZD-1, D937, 8112 and 835 were sensitive to low K stress. This study provides the important germplasm resources for genetic improvement of K efficiency in maize breeding program.

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1 Introduction

Potassium (K) is one of the essential nutrients for crop growth, which plays an important role in crop metabolism, development and final yield increasing process (Mengel, 1985; Marschner, 2012). In recent years, about 60% of soil are showing K deficiency (available K content less than 70mg/kg) as a result of long-term agricultural production of existing cultivated land in China, could not meet the needs of crop yield improvement (Yang et al., 2004; Pettigrew, 2008; Jin, 2012). Additionally, higher K deficiency was reported in acid sandy, waterlogged and saline soils (Mengel & Kirkby, 2001). Meanwhile, the price of K fertilizers rapidly increased with raising the prices of energy. Maize is an important crop and need more K. Currently, the amount of potash fertilizer applied to meet the demand of corn is 1/5-1/3, and it has limiting effect on the improvement of yield and quality of maize (Römheld & Kirkby, 2010; Jin, 2012). Thus, it is clear that improving the efficiency of K absorption from soil should have benefit in the economic and environmental by cultivating crop genotypes that acquire and utilize K more effectively (Rengel & Damon, 2008; Fageria et al., 2010; White & Brown, 2010).

Root morphological characters, including the length, the number, orientation, surface area, volume, and diameter, influence the absorption of mineral nutrients from soil and increase yield of crops (Lynch, 2005; Bellini et al., 2014). The root morphology is highly fitile and differ among intraspecific and species level, which adapts to the variations in nutritional concentration to optimize uptake (Niu et al., 2013). Some reports indicated that the crop varieties resistant to low K adjusted the root morphology and active absorption ability to adapt to low K stress (Fageria et al., 2001; George et al., 2002).

The total amount of K absorbed by the plant was compositely depended on the crop species, the availability of K in the soil, the amount of K fertilizer applied, the growth conditions during the growing season and the management practices employed (Pettigrew and Meredith, 1997; Mullins & Burmester, 1998). The majority of K uptake in soybean occurs after flowering (Oosterhuis et al., 2013). Similarly, cotton also absorbs the majority of K at the blooming and boll-filling period (Mullins & Burmester, 1990). On the other hand, most of K uptake in maize takes place before silking (Karlen et al., 1988).

Research showed that maize seedlings were sensitive to K deficiency (Cao et al., 2007). In this study, 15 maize inbred lines were used as test materials, field experiments conducted in natural soil K, the morphology of root system and dry matter accumulation, plant K accumulation changes response to the low K stress were investigated, specifically the screening inbred lines of maize tolerance to low K deficiency at seedling

stage could provide the germplasm resources for genetic improvement of tolerance to K deficiency.

2 Materials and Methods

Fifteen maize inbred lines i.e. 90-21-3, 91-2, D1029-2B, 099, D937, N40, 8112, 835, Q319, Z-58, LD9801, CQ-2, LD9-1, ZD-1 and ZD-2 were planted on 11 May in 2012 and on 15 May in 2013. The study was carried out in an experiment field unapplied K fertilizer from last 5 years located in Manduhu rural (41°32'N, 122°43'E), Shenyang City, Liaoning Province, China. The amount of nutrition elements were measured by using the method described by Alban & Kellogg (1959). The initial soil contained 65.5 mg/kg available K, 87.3 mg/kg alkaline hydrolysis N, Olsen-P 43.1 mg/kg. Low K (-K) and high K (+K) treatments were implemented.

The K fertilizer was not applied in -K treatment, while sulfuric acid K fertilizer @ 150kg/ha was applied and adjusted K content to 130mg/kg on +K treatment. In addition, DAP (Diammonium phosphate) @ 150 kg/ha and urea @ 150 kg/ha were applied as a types of fertilizer. 8 rows per inbred with line spacing 0.60m, length with 10m were cultivated on each experimental plots. The hole spacing was 0.30m. The experiment was designed in Randomized block design and each treatments were replicated with three times.

At the four-leaf stage, 3 representative samples were collected to measure the root morphology by using WinRHIZO Program (Regent Instruments Inc., Canada), including total root length, root surface area, root volume and average root diameter. From a square of soil block (30cm×30 cm), the base of plant was dug and 3 whole roots system were used to measure. The parts of root and shoot were placed in brown paper bags, and stored at 105°C high temperature for 1h, then weighed after drying to constant weight at 80 °C. K content of plants was extracted by H₂SO₄-H₂O₂ digestion and measured with flame spectrophotometry (Sherwood M410 flame photometer, England) (Miller, 1998).

$K \text{ accumulation (mg)} = K \text{ content (mg/g)} \times \text{dry weight (g)}$

3 Results

3.1 The variation of root morphology traits

The significant F tests for root morphology treats for 15 selected maize inbred lines were shown in Table 1. Although the interaction of genotype × K level for total root length was not significant but the total root length, root surface area, root volume and root average diameter were significant or highly significant difference among various inbred lines.

Table 1 Variance analysis for root morphological traits in 15 maize inbred lines (F value).

Source of variation	DF	Total root length	Root surface area	Root volume	Root average diameter
Genotype	14	159.019**	198.643**	345.687**	28.065**
K level	1	11.647**	230.806**	295.35**	50.562**
Genotype×K level	14	1.276ns	9.811*	12.788**	12.053**
Error	30	161.725	11.409	0.002	0.001

Note: * and ** stands for the significant levels at 0.05 and 0.01, respectively, ns stand for non significance, value give in table are mean of three replicates.

Table 2 shows the variations in root morphological traits for different maize inbred lines in positive and -K treatments. Under -K treatment, most inbred lines except Q319, ZD-1, 8112 shown the trend of lengthening compared to +K treatment. The total root length of 90-21-3, ZD-2, 099 and 91-2 were highly increased by 6.13%, 7.21%, 11.83% and 10.85% compared to +K treatment, respectively. The total root lengths of other inbred lines were slightly increased, but no significant difference compared to +K treatment.

The root surface area of Q139, Z-58, LD9801, LD9-1, ZD-2, 90-21-3, 91-2 and 099 were slightly decreased 1.91% ~ 11.82% compared to +K treatment, whereas the root surface

area of other inbred lines were significantly decreased. It is worthy note that the root surface of CQ-2, ZD-1 and 835 were significantly decreased by 31.17%, 39.59% and 25.92% compared to +K treatment, respectively.

The root volume of inbred lines, including CQ-2, D937, 8112, and 835, were significantly decreased 20.18%, 25.33%, 21.32% and 34.17% compared to +K treatment, respectively. Other inbred lines showed little variation under -K treatment. The root average diameter of ZD-1 was significantly increased under -K treatment, while the other inbred lines were decreased compared to +K treatment, especially ZD-2 decreased 38.53%.

Table 2 Effect of different K treatments on various root traits for selected fifteen maize inbred lines.

Inbred lines	Total root length (cm)			Root surface area(cm ²)			Root volume(cm ³)			Root average diameter(mm)		
	+K	-K	±%	+K	-K	±%	+K	-K	±%	+K	-K	±%
Q319	333.55	332.92	-0.12	91.04	82.06	-9.86	1.83	1.62	-11.48	0.87	0.79	-9.20
Z-58	452.68	460.06	1.63	96.49	93.03	-3.59	1.31	1.26	-3.82	0.69	0.66	-4.35
LD9801	412.74	418.64	1.43	108.04	97.30	-9.94	1.85	1.61	-12.97	0.69	0.66	-4.35
CQ-2	316.58	321.63	1.60	65.22	44.89	-31.17	1.09	0.87	-20.18	0.68	0.65	-4.44
LD9-1	330.12	336.48	1.93	59.79	54.10	-9.52	0.97	0.95	-2.06	0.71	0.70	-1.41
ZD-1	397.45	392.01	-1.37	104.24	62.97	-39.59	2.14	2.04	-4.67	0.83	0.85	2.41
ZD-2	170.81	183.12	7.21	43.82	38.64	-11.82	0.61	0.59	-3.28	1.09	0.67	-38.53
D937	279.09	287.23	2.92	75.69	65.53	-13.42	1.50	1.12	-25.33	0.75	0.69	-8.00
N40	494.79	504.01	1.86	141.71	122.87	-13.30	2.37	1.98	-16.46	0.89	0.85	-4.49
8112	377.10	373.83	-0.87	74.81	63.28	-15.41	1.36	1.07	-21.32	0.69	0.66	-4.35
835	414.94	418.31	0.81	114.08	84.51	-25.92	1.99	1.31	-34.17	0.70	0.61	-12.86
90-21-3	397.43	421.81	6.13	105.69	97.48	-7.77	2.14	1.99	-7.01	0.79	0.78	-1.27
91-2	321.29	359.31	11.83	100.60	90.66	-9.88	1.75	1.61	-8.00	0.71	0.71	0
D1029-2B	421.58	433.18	2.75	110.14	96.60	-12.29	1.63	1.39	-14.72	0.67	0.64	-4.48
099	420.93	466.62	10.85	68.10	66.80	-1.91	1.62	1.53	-5.56	0.73	0.71	-2.74
Mean	369.41	380.61	-	90.63	77.38	-	1.61	1.40	-	0.77	0.71	-
SEM	20.60	20.97	-	6.60	5.97	-	0.13	0.11	-	0.03	0.02	-

Value give in table are mean of three replicates

Table 3 Variance of analysis for dry matter weight in 15 maize inbred lines (F value).

Source of variation	DF	Root dry weight	Shoot dry weight	Root to shoot ratio
Genotype	14	12.377**	73.814**	8.327**
K level	1	153.938**	116.59**	27.013**
Genotype×K level	14	4.894**	6.485**	1.926ns
Error	30	0.001	0.003	0.002

Note: * and ** stands for the significant levels at 0.05 and 0.01, respectively, ns stand for non significance, value give in table are mean of three replicates.

Table 4 Effect of different K treatments on dry matter weight and root to shoot ratio in 15 maize inbred lines.

Inbred lines	Root dry weight			Shoot dry weight			Root to shoot ratio		
	+K	-K	±%	+K	-K	±%	+K	-K	±%
Q319	0.32	0.22	-31.25	0.99	0.75	-24.24	0.32	0.29	-9.38
Z-58	0.26	0.25	-3.85	0.68	0.61	-10.29	0.38	0.41	-7.89
LD9801	0.48	0.22	-54.17	1.40	1.35	-3.57	0.34	0.16	-5.29
CQ-2	0.27	0.18	-33.33	0.80	0.78	-2.50	0.34	0.23	-3.24
LD9-1	0.25	0.23	-8.00	0.71	0.71	0	0.35	0.32	-8.57
ZD-1	0.43	0.21	-51.16	1.02	0.54	-47.06	0.42	0.39	-7.14
ZD-2	0.16	0.14	-12.50	0.70	0.65	-7.14	0.23	0.22	-4.35
D937	0.42	0.17	-59.52	1.03	0.74	-28.16	0.41	0.23	-43.90
N40	0.39	0.26	-33.33	1.19	0.82	-31.09	0.33	0.32	-3.03
8112	0.29	0.21	-27.59	0.95	0.69	-27.37	0.31	0.30	-3.23
835	0.33	0.20	-39.39	0.74	0.62	-16.22	0.44	0.32	-27.27
90-21-3	0.36	0.28	-22.22	1.04	1.02	-1.92	0.35	0.27	-22.86
91-2	0.29	0.23	-20.69	1.06	0.99	-6.60	0.27	0.23	-14.82
D1029-2B	0.33	0.23	-30.30	1.55	1.41	-9.03	0.22	0.16	-27.27
099	0.47	0.36	-23.40	1.03	1.00	2.91	0.46	0.36	-21.74
Mean	0.34	0.23	-	0.99	0.85	-	0.34	0.28	-
SEM	0.02	0.01	-	0.07	0.07	-	0.02	0.02	-

Value give in table are mean of three replicates

3.2 The variation of dry matter accumulation

The significant F test for dry matter accumulation for 15 inbred lines is shown in Table 3. The variations in dry root and shoot weight were highly significant among various genotypes and K levels and the interaction of genotype×K level. However, the root to shoot ratios were highly significant different among genotypes and K levels, whereas the interaction of genotype×K levels were not significant.

Table 4 shows the changes in dry matter accumulation and the ratio of root to shoot among different inbred lines between two treatments. Under -K treatment, the root dry weight, shoot dry weight and root to shoot ratio were decreased at varying degrees.

In case of -K treatments, the root dry weight of Q319, CQ-2, N40, 8112, D1029-2B and 835 were decrease 27.59% ~ 39.39% as compared to +K treatment, while those of LD9801, ZD-1 and D937 were highly decreased 54.17%, 51.16% and 59.52%, respectively. Comparing with +K treatment, the shoot dry weight of Q319, Z-58, ZD-1, D937, N40, 8112 and 835 were decreased in -K treatment, while there were not significant for other inbred lines.

The root to shoot ratio of D937 and 835 were significantly affected by low K stress, whereas the other inbred lines had no significant effect under -K treatment. In case of dry matter accumulation indexing, the inbred lines, LD9-1, ZD-2, 91-2, 099 and 90-21-3, were less affected by the low K stress, but ZD-1, D937, 8112, 835 were more sensitive to low K deficiency.

Table 5 Variance analysis of KAA in 15 maize inbred lines (F value).

Source of variation	DF	KAA of root	KAA of shoot	KAA of plant
Genotype	14	12.377**	73.814**	8.327**
K level	1	153.938**	116.59**	27.013**
Genotype X K level	14	4.894**	6.485**	1.926ns
Error	30	0.001	0.003	0.002

Note: * and ** stands for the significant levels at 0.05 and 0.01, respectively, ns stand for non significance, value give in table are mean of three replicates.

Table 6 Effect of different K treatments on KAA in 15 maize inbred lines.

Inbred lines	KAA of root			KAA of shoot			KAA of plant			
	+K	-K	±%	+K	-K	±%	+K	LSR _{0.05}	-K	LSR _{0.05}
Q319	2.87	2.62	-8.71	21.17	15.52	-26.19	24.04	de	18.13	b
Z-58	2.38	1.42	-40.34	10.41	6.79	-34.77	12.79	f	8.22	e
LD9801	5.38	2.36	-56.13	26.34	20.57	-21.91	31.72	cd	22.93	a
CQ-2	3.37	1.10	-67.36	26.97	16.28	-39.64	30.34	cd	17.39	bc
LD9-1	1.76	1.33	-24.43	10.35	9.61	-7.15	12.10	f	10.94	de
ZD-1	3.61	1.06	-70.64	14.78	8.71	-41.07	18.39	e	9.76	e
ZD-2	1.95	1.55	-20.51	10.23	12.18	19.06	12.17	f	13.72	cd
D937	8.85	1.27	-85.65	31.28	17.96	-42.58	40.13	B	19.23	b
N40	6.16	2.10	-65.91	34.85	13.03	-62.61	41.01	B	15.13	c
8112	5.48	1.51	-72.45	26.50	10.99	-58.53	31.97	Cd	12.50	d
835	5.90	2.00	-66.10	26.40	8.80	-66.67	32.30	Cd	10.80	de
90-21-3	6.81	4.21	-38.18	31.75	19.82	-37.57	38.55	B	24.03	a
91-2	6.90	2.45	-64.49	32.19	15.05	-53.25	39.09	B	17.50	b
D1029-2B	6.83	2.01	-70.57	56.28	21.86	-61.16	63.11	A	23.87	a
099	6.95	4.01	-42.30	30.17	18.42	-38.95	37.12	Bc	22.43	a
Mean	5.01	2.07	-	25.98	14.37	-	30.99	-	16.43	-
SEM	0.57	0.25	-	3.09	1.23	-	3.54	-	1.38	-

value give in table are mean of three replicates

3.3 The variation of K accumulation amount (KAA)

The significant F test of 15 inbred lines for KAA is shown in table 5. Different genotypes, different K levels and the interaction of genotype × K level were all highly significant, which indicated different genotype of inbred lines showed various abilities under the low K stress conditions.

Table 6 shows the variations for KAA in different inbred lines between two treatments. Under the K stress conditions, the KAA of root, shoot and whole plant in most inbred lines, except ZD-2 for shoot and whole plant, were decreased. KAA of roots were decreased from 8.71% (Q319) to 85.65% (D937). The inbred lines, including ZD-1, D937, 8112 and D1029-2B, were significantly differing between -K and +K treatments. Comparing with +K treatment, KAA in shoot of in N40, 8112, 835, 91-2, D1029-2B, were significant decreased in -K treatment. Under K stress, KAA of plant in LD9801, 90-21-3,

D1029-2B and 099 were significantly higher than those in other inbred lines. However, KAA of plant in Z-58, LD9-1, ZD-1, 835 were significantly lower than other inbred lines.

Discussions

Plant absorbed mineral nutrition mainly by the root system. In response to the nutrition deficiency, plant has developed various strategies to adaptively the stress by changing root system architecture (Drew, 1975; Ma et al., 2014). Cao et al. (2007) reported that maize inbred lines tolerant the K deficiency and adjusted the root morphology with H⁺/K⁺ exchange ability to improve absorption capacity under K stress in order to maintain normal growth. A series of ideotype root that enhance topsoil foraging such as shallow axial root growth angles, long root hairs also enhance P acquisition, and are now being utilized on crop breeding programs for stressful soil environments (Lynch, 2013). The results of the present study

showed that maize root morphology and dry matter accumulation were significantly affected under K stress. The inbred lines of tolerance to K deficiency have longer roots, while the root volume is relatively reduced and the root became more delicate. The total root length, root surface area, root volume and average root diameter were significant or highly significant among various genotypes. Thus, under K stress, the root morphology of maize showed different degrees of change, mainly in longer total root length. Rengel & Damon (2008) considered that genotypes efficient in K uptake have a larger surface area of contact between roots and soil and maintain a larger diffusive gradient towards roots in crop. In addition, the adaptations of the root system to variations in N supply were summarized with four strategies on lateral root development, including the localized stimulatory effect of external nitrate, the systemic inhibitory effect of high tissue nitrate concentrations, the suppression effect of high C:N ratios and the inhibition and stimulation effect of external L-glutamate (Zhang et al., 2007).

K is necessary for crop growth and productivity and occupies 2-5% of the total plant dry weight (Marschner, 2012). The decrease of carbohydrate accumulation is obvious symptom under K deficiency conditions in cotton, which is attributed in decreased efficiency of abundant enzymes (Bednarz & Oosterhuis, 1999). The highly significant difference of root dry weight, shoot dry weight and root to shoot ratio were observed among genotypes, K levels. In present study, the dry weight of LD9-1, ZD-2, 91-2, 099 and 90-21-3 were slightly decreased under -K treatment, whereas those of ZD-1, D937, 8112, 835 were largely decreased. Korir et al. (2010) considered that the total plant dry weight was an appropriate representative indicator for Al tolerance evaluation due to easy to measure in soybean. These results indicated that the variations of root and shoot dry weight should be as directly and appropriate indicator for K tolerance identification in maize.

The ability of roots to acquire K from the soil is referred to as K uptake efficiency. Different genotype species differ during their growth period, their ability to absorb from soil and their ability to utilize K physiologically for vegetative and reproductive growth (Grant et al., 2007; Samal et al., 2010; Hafsi et al., 2011). Better K acquisition ensures the physiological K requirement for crop under K deficiency soil, while better physiological utilization produces more at a lower K supply condition (White, 2013). Accumulation of KAA in plant can directly reflect the ability of plants to absorb K from soil, so K uptake ability of maize inbred lines tolerance to K deficiency was also high. The ability of roots absorbing K at a higher rate has a significant potential to influence efficiency of K uptake under K deficiency stress (Römhald & Kirkby, 2010). In present study, four inbred lines had longer root, more dry matter and higher KAA, which indicated maize inbred lines tolerance to K deficiency could absorb and effectively utilize K to maintain normal growth and development of plant.

In a word, root morphology of maize characteristics, dry matter accumulation and K accumulation were significant differences among genotypes under K deficiency. We conclude that ZD-2, 91-2, 099 and 90-21-3 were relatively high resistance to low K, while ZD-1, D937, 8112, and 835 were more sensitive to K deficiency.

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