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Full Length Research Paper

Some physiological characteristics in maize (*Zea mays* L.) inbred lines tolerant to low potassium from grain filling to maturity

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Northeast China produces an abundance of maize (*Zea mays* L.). 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 (90-21-3) and sensitive to K deficiency (D937) were grown in potassium deficiency field conditions. We investigated the root morphology, leaves stay-green, K centent, K utilization efficiency (KUE), yield from grain filling to maturity. We found that the responses to low potassium stress of 90-21-3 mainly embodied in root dry matter accumulation, total root length, root volume, root surface area and average diameter changed more slightly than D937; the leaf stay-green in 90-21-3 were better than that in D937; Chlorophyll content increased to some extent in 90-21-3, while it decreased sharply in D937; K content in root, leaf, ear increased slightly in 90-21-3, but the K content of each organ in D937 were decreased much more; K content of each leaf and leaf sheath in 90-21-3 declined much smaller than D937, particularly where it found in three ear-leaves and sheaths; ear dry weight and yield of 90-21-3 decreased less than D937; K use efficiency of 90-21-3 was higher than D937. Under low potassium stress, the root system of 90-21-3 was relatively strong enough to absorb K, moreover the K contents in three ear leaves and sheaths were maintained. The leaf stay-green could prolong photosynthetic period. Thus, dry matter accumulation and yield were relatively higher than that of D937.

Key words: Maize (*Zea mays* L.) tolerant to K deficiency, grain filling to maturity stage, root morphology, K absorption, dry weight, leaf stay-green, yield.

INTRODUCTION

Global potassium (K) reserves are being depleted and commercially viable reserves of potassium mineral resources are being used so rapidly that these will be exhausted within the next 25 to 100 years (Kesler, 2007). Potassium (K) is an essential macronutrient required for Maize (*Zea mays* L.) to complete life cycle. It fulfills important functions and it is widely used as a fertilizer to increase crop production. Potassium deficiency in the northern region of China reduces maize growth and crop yields. Moreover, the shortage of organic fertilizer caused the continued lack of potassium in local soil. K increases crop production alone or in a synergistic effect with other macroelements and microelements (Orosz et al., 2009; Losak et al., 2010, 2011). It has been proposed that crops production utilizes K more effectively can reduce the use of expensive K fertilizers in agriculture (Rengel and Damon, 2008). The excessive use of fertilizers in agriculture is a

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major contributor to eutrophication processes in waters of both developed and developing nations (Conley et al., 2009; White and Hammond, 2009). For both commercial and environmental reasons, it is clear that using crop's own biological characteristics to adapt low potassium stress is a feasible way of potassium efficiency utilization. The visual symptoms of K deficiency appeared mainly as old leaves chlorose, in addition, filling to maturity stage is a critical period of maize yield formation.

Stay-green is the capacity to maintain green leaves (Thomas and Smart, 1993) and is considered to be effective in reducing the influence of drought in sorghum (Borrell et al., 2000a, 2000b, 2001; Borrell and Hammer, 2000; Mahalakshmi and Bidinger, 1985) and in wheat (Christopher et al., 2008). While what relationship existing between K content and leaves chlorose is still not clear. Mineral elements are acquired from soil solution by plant roots, so does potassium. Root systems serve many important tasks among which anchorage of the plant and uptake of water plus nutrients are the most important ones (Aiken and Smucker, 1996). The absorption and distribution of potassium nutrition within the plant have been the subject of studies for many decades (Mengel et al., 2001; Karley and White, 2009; Miller et al., 2009). There is a need to develop crops that are highly effective at acquiring potassium nutrition from K-deficiency soils. Root morphology in soil has always been a research goal as it may eventually produce high nutrition absorption to enhance crop productivity further. In contrast to previous assessments which focused on the early growth stage with pot and water culture experiments, this paper are emphasizing on the grain filling to maturity stage through field trials in natural K deficient soil. This paper will propose to verify the features of maize inbred lines tolerant to low potassium stress including root morphology, leaf stay-green, K absorption, dry weight, yield and K utilization efficiency (KUE).

The results could be beneficial to reveal the physiological mechanism and breed new variety of resistant to potassium deficiency in maize.

MATERIALS AND METHODS

The experiment was conducted in Manduhu Town (41° 32′ N, 122° 43′ E), Liaozhong County, Shenyang City, Liaoning Province in 2009 and 2010. The soil type was sandy and lack potassium. The soil indexes were determined following the procedures outlined by Alban and Kellogg (1959). The initial soil contained these amounts of elements: available K (flame photometer method) 48.3 mg/kg, alkaline hydrolysis N (Kjeldahl method) 87.3 mg/kg soil, Olsen-P (sodium bicarbonate method) 43.1 mg/kg soil, pH (soil: water ratio of 1:1) was 7.1. Soil samples are air-dried and then passed through a mechanical crusher consisting of two rollers which turn toward each other. The soil then passes through a 10-mesh screen. The rollers are kept clean by large brushes held tightly against them. The soil samples are kept for at least 5 days before being tested, especially for potassium. The test maize inbred lines 90-21-3 (indicated by T in figures) was from Ludahongcob group and the inbred line D937 (indicated by S in figures) was from Reid group. Experimental design had two treatments, the low potassium (-K), potash feritilizer was not applied on the test field; high potassium (+K), sulfuric acid potassium fertilizer of 150 kg/ha was applied on the test field which was according to the fertilizer application of local farmers commonly used. 8 rows spacing 0.60 m, length with 10 m were arranged on each experiment plots. Hole spacing was 0.30 m. Randomized block design with three replications was used. In the experiment of 2009-2010, maize was sown on 5th May and harvested on 27th September in 2009. Maize was sown on 2nd May and harvested on 28th September, 2010.

In addition to the application of potash fertilizer treatment, diammonium phosphate and urea 150 kg/ha were used as seed fertilizer, both in 90-21-3 and D937. At spike formation stage, 315 kg/ha urea were applied as topdressing. Other field management were the same with routine maize production field.

Root sampling and measurements

3 representative plants were selected in each plot. To the average of the three times as a repeat, 3 repeats were conducted. A square block of soil (100 × 100 cm) with the plant base at the center was dug to reach the end of tap root to get the complete 3 plants root system in each plot. In the field, root architecture was classified based on the initial growth angle of basal roots as described earlier (Liao, 2000; Liao et al., 2001). The roots were carefully cleaned before being scanned into the computer and the digital images were quantified by the scanner specially for getting data of root systems with computer image analysis software (Win-RHIZO 2.0 Pro2005, Régent Instruments, Québec, Canada) for root morphological parameters such as total root length (cm), root surface area (cm^2), root volume (cm^3), average diameter (mm) and other indicators.

Dry matter accumulation

Each leaf, stem, tassel, ear and roots were dried at 105°C for 30 min, then kept at 75°C until completely dry (about 2 days). The determination was according to the method described by JCS Allison and Watson (1966).

Leaves stay-green

Stay green was measured according to the method by Haussmann et al. (2002) and Tinker (1984). Identification of cell in the middle of each of 5 randomly selected plant listed markers. In flowering and grain filling stage (20 days after flowering) to investigate different inbred lines of green leaf area per plant for each listing (length × width × 0.75). Green leaf area duration (GLAD) has been associated frequently with yield. Absolutely, GLAD is measured according to the formula that multiply green leaf area at flowering stage by 20 days. Relative green-leaf area (RGLA) is the ratio of the green leaf area at grain filling stage to the green leaf area at flowering stage. The number of green leaves were measured, the green leaf area ratio was greater than 50%.

Chlorophyll content

We used ethanol and acetone method (Dos Santos et al., 2003) to determine on the chlorophyll content. Using the formula, chlorophyll a = $12.7A_{663}$ to $2.59A_{645}$, chlorophyll b = $22.9A_{645}$ to $4.67A_{663}$ and chlorophyll (a+b) = $20.3A_{645}$ to $8.04A_{663}$.

Table 1. Analysis of variance of the effect of inbred lines and K levels on dry matter accumulation of each organ across 2 years.

SOV	df	Shoot		Root		Stem		Leaf		Ear		Spike	
		F.	Sig.	F.	Sig.	F.	Sig.	F.	Sig.	F.	Sig.	F.	Sig.
Year	1	0.09	0.77	2.83	0.11	0.09	0.76	0.09	0.76	0.09	0.77	0.08	0.78
K	1	280.08**	0.00	227.39**	0.00	97.41**	0.00	24.90**	0.00	578.54**	0.00	721.43**	0.00
Lines	1	67.61**	0.00	214.28**	0.00	27.35**	0.00	224.05**	0.00	64.28**	0.00	49.10**	0.00
Year × K	1	0.00	0.96	0.04	0.84	0.00	0.97	0.00	0.99	0.01	0.94	0.01	0.93
K × lines	1	72.88**	0.00	34.71**	0.00	71.16**	0.00	3.23	0.09	137.15**	0.00	349.12**	0.00
Year × lines	1	0.00	0.98	1.85	0.19	0.00	0.99	0.00	0.96	0.00	0.98	0.00	0.98
Year × K × lines	1	0.00	0.98	0.02	0.90	0.00	0.98	0.00	1.00	0.00	0.97	0.00	0.95

*, ** indicated the difference at 0.05, 0.01 levels, respectively.

K content

Grinding mill prototype of each leaf, stem, tassel, ear and roots, with the H_2SO_4-H_2O_2 digestion (Miller, 1998), using flame photometer (Sherwood M410 flame photometer, England). Using the formula K accumulation (mg) = K content (mg/g) × sample dry weight (g) calculation of plants in different parts of potassium accumulation.

Observed yield and yield components

Grain yield per plant (g) harvested at maturity, the full ear of each experimental plot, threshing and drying (moisture content 14%), grain weight, weighed, divided by the number of plants of each experimental plot; grain weight (g), taking the 1000 air-dried grain weighing was repeated three times; corn ear weight, uniform selection of the ear area with 1/1000 scale weighed, and measured spike length (cm), bare top length (cm) was repeated 10 times averaged. The determinations were according to the method that was described.gu

Potassium utilization efficiency (KUE)

Grain K utilization efficiency (KUE) = grain yield (g)/whole plant K accumulation (mg), relative potassium utilization efficiency (RKUE) = K utilization efficiency under low potassium/potassium utilization efficiency under high K, straw K utilization efficiency = straw dry weight (g)/whole plant K accumulation (mg), plant K utilization efficiency = dry weight (g)/whole plant K accumulation (mg). The methods were discribed by Liu et al. (2000).

Statistical analyses

Data collected were subjected to analysis of variance (ANOVA) to determine the effects of inbred line, K level, year and their interactions on each phenotypic trait. Statistical analysis were performed with the statistical program SPSS for Windows 18.0.

RESULTS

Dry matter accumulation

The interaction of year with both K and inbred lines was not significant across years (Table 1). In addition, K × inbred lines were statistically significant for dry matter of each organ. Table 2 showed the dry matter of two inbred lines under different application of K. As a whole, 90-21-3 declined lighter in dry weight than D937 under low potassium stress. Root dry weight of 90-21-3 decreased by 13.83 and 10.07% respectively under low potassium stress in 2009 and 2010, and they decreased by 2 times more in D937. The difference in D937 between two treatments reached a significant level. The shoot dry weight showed a marked reduction in D937, differed significantly between +K and -K in two years, but a slight decline in 90-21-3. Ear, spikes, stem and leaves dry weight in D937 declined significant reductions between two treatments. They decreased in D937 much more than that in 90-21-3. The responses of ear and leaves were more susceptible in D937.

Root morphology

Careful examination of the data seems to indicate that there may be a statistically significant difference in two inbred lines on the root morphological parameter of the plants from the two K treatments (Table 3). D937 was shorter in root length under low potassium stress but 90-21-3 was a little longer than +K treatment. In 2009 and 2010, the root lengths of D937 declined by 39.53 and 28.10% respectively, relative to the +K treatment, while they increased by 1.62 and 2.74% in 90-21-3 (Table 4). The volume of root in low potassium conditions exhibited shrinkage in two inbred lines. But the decline in D937 was 4 times more than that in 90-21-3. Root surface area showed a similar change with root volume under low potassium stress. They decreased by 18.65 and 8.14% in 90-21-3 in 2009 and 2010, and decreased by 21.26 and 19.01% in D937. The root average diameter decreased in both two inbred lines under K-deficient conditions, with extremely significant difference between two treatments. However, they decreased much more in the D937 compared with the 90-21-3. In 2009, 90-21-3 decreased by 2.22% and D937 decreased 14.29%, the similar results showed in 2010.

Table 2. Effects of two K levels on dry matter accumulation in 90-21-3 and D937 across 2 years.

Inbred line	Treatment	Root (g/plant)		Shoot (g/plant)	
		2009	2010	2009	2010
	+K	24.44	23.24	235.33	223.84
90-21-3	-K	21.06	20.90	191.09	189.83
	±%	-13.83	-10.07	-18.80	-15.19
	+K	20.81	21.67	226.64	215.15
D937	-K	13.33	13.24	121.10	113.30
	±%	-35.94	-38.90	-46.57	-47.34
Inbred line	**Treatment**	**Ear (g/plant)**		**Spikes (g/plant)**	
		2009	**2010**	**2009**	**2010**
	+K	118.68	117.89	3.84	3.61
90-21-3	-K	92.01	90.40	3.13	3.11
	±%	-22.47	-23.32	-18.49	-13.85
	+K	126.65	125.82	6.06	6.02
D937	-K	49.39	46.06	2.12	2.11
	±%	-61.00	-63.39	-65.02	-64.95
Inbred line	**Treatment**	**Stem (g/plant)**		**Leaves (g/plant)**	
		2009	**2010**	**2009**	**2010**
	+K	54.44	59.10	43.33	42.04
90-21-3	-K	51.06	57.39	38.18	37.93
	±%	-6.21	-2.89	-11.89	-9.78
	+K	60.81	62.91	38.60	37.40
D937	-K	43.33	41.14	28.18	27.99
	±%	-28.75	-34.60	-26.99	-25.16

Table 3. Analysis of variance of the effect of inbred lines and K levels on root morphological parameters across 2 years.

SOV	df	Root length		Root volume		Root surface area		Root average diameter	
		F.	Sig.	F.	Sig.	F.	Sig.	F.	Sig.
K	1	7.51*	0.02	12.46**	0.00	303.00**	0.00	2.62	0.13
Lines	1	5.29*	0.04	113.79**	0.00	13.19**	0.00	11.08**	0.00
Year	1	0.00	1.00	0.13	0.73	162.13**	0.00	0.13	0.73
Lines × year	1	4.56	0.05	1.17	0.30	0.22	0.64	2.49	0.13
K × lines	1	8.93*	0.01	96.82**	0.00	0.02	0.90	10.99**	0.00
K × year	1	0.15	0.70	0.99	0.34	59.12**	0.00	0.04	0.84
K × lines × year	1	1.28	0.27	2.16	0.16	0.00	0.97	1.83	0.20

*, ** indicated the difference at 0.05, 0.01 levels, respectively.

Leaf stay-green

The K levels significantly affected the number of stay-green leaves, green leaf area, green leaf area duration (GLAD) and relative green leaf area (RGLA) (Table 5). What is more, the difference between two inbred lines was significant across two years. Significant differences between K levels were found for the interaction

Table 4. Effects of two K levels on root morphological parameters in 90-21-3 and D937 across 2 years.

Inbred line	Treatment	Total root length (cm)		Root volume (cm^3)		Root surface area (cm^2)		Root average diameter (mm)	
		2009	2010	2009	2010	2009	2010	2009	2010
90-21-3	+K	7770.15	7483.82	2426.28	2377.75	63.48	62.06	0.90	0.89
	-K	7895.64	7688.73	2216.67	2074.34	51.64	57.01	0.88	0.79
	±%	1.62	2.74	-8.64	-12.76	-18.65	-8.14	-2.22	-11.24
D937	+K	7623.02	8272.71	1731.70	1697.07	44.01	42.36	0.77	0.90
	-K	4609.69	5948.35	887.63	850.28	34.65	34.30	0.66	0.67
	±%	-39.53	-28.10	-48.74	-49.90	-21.27	-19.03	-14.29	-25.56

Table 5. Analysis of variance of the effect of inbred lines and K levels on leaf stay-green characters across 2 years.

SOV	df	Green leaf number		Green leaf area		GLAD		RGLA	
		F.	Sig.	F.	Sig.	F.	Sig.	F.	Sig.
Year	1	1.38	0.26	0.55	0.47	1.34	0.26	2.47	0.14
K	1	60.48**	0.00	33.91**	0.00	1321.20**	0.00	2037.40**	0.00
Lines	1	144.46**	0.00	78.35**	0.00	517.90**	0.00	69.98**	0.00
Year × K	1	0.01	0.91	1.75	0.21	0.14	0.72	0.21	0.66
K × lines	1	12.49**	0.00	5.46*	0.03	0.44	0.52	356.54**	0.00
Year × lines	1	0.03	0.86	0.95	0.34	0.05	0.82	0.01	0.93
Year × K × lines	1	0.03	0.96	0.01	0.91	0.00	1.00	0.04	0.85

*, ** indicated the difference at 0.05, 0.01 levels, respectively.

Table 6. Effects of two K levels on leaf stay-green characters in 90-21-3 and D937 across 2 years.

Inbred line	Treatment	The number of stay green leaves			Leaf area per plant (cm^2)		
		2009	2010	Mean	2009	2010	Mean
90-21-3	+K	11.00	11.00	11.00	4258.33	4173.16	4215.75
	-K	9.00	10.00	9.50	3478.22	3408.66	3443.44
D937	+K	9.00	10.00	9.50	3053.11	2992.05	3022.58
	-K	6.00	6.00	6.00	1568.29	1536.92	1552.61

Inbred line	Treatment	GLAD ($m^2 \cdot d$)			RGLA (%)		
		2009	2010	Mean	2009	2010	Mean
90-21-3	+K	14.97	14.67	14.82	85.86	81.53	23.695
	-K	8.80	8.62	8.71	68.72	61.14	19.86
D937	+K	11.15	10.93	11.04	88.72	81.43	30.06
	-K	4.75	4.66	4.71	41.31	43.75	12.53

of K and inbred lines. K-deficient treatment decreased green leaf numbers in comparison with the +K treatment (Table 6). Similarly, green leaf area in K-deficient conditions was smaller than that of the +K treatment throughout the experimental period. However, the decline in 90-21-3 were much smaller than that in D937 due to K deficiency. GLAD and RGLA showed a similar trend under low potassium stress.

Chlorophyll content

First sign of potassium deficiency is the loss of the normal green color at the leaf tips. It is directly bound up with

Table 7. Analysis of variance of the effect of inbred lines and K levels on chlorophyll content across 2 years.

SOV	df	Chlorophyll a		Chlorophyll b	
		F.	Sig.	F.	Sig.
Year	1	0.46	0.51	0.09	0.77
K	1	0.29	0.60	5.97*	0.03
Lines	1	25.71**	0.00	22.12**	0.00
Year × K	1	0.00	0.99	0.00	0.97
K × lines	1	74.09**	0.00	37.80**	0.00
year × lines	1	0.01	0.93	0.01	0.94
year × K × lines	1	0.02	0.88	0.01	0.92

*, ** indicated the difference at 0.05, 0.01 levels, respectively.

Table 8. Effects of two K levels on chlorophyll content in 90-21-3 and D937 across 2 years.

Inbred lines	Treatments	Chlorophyll a (mg/g)		Chlorophyll b (mg/g)	
		2009	2010	2009	2010
90-21-3	+K	0.80	0.81	1.12	1.23
	-K	0.81	0.81	1.16	1.25
D937	+K	0.86	0.82	1.19	1.16
	-K	0.83	0.82	0.99	1.08

chlorophyll degradation. As Table 7 displays, the difference of chlorophyll contents in two inbred lines between two K treatments reached an extreme significant level. The chlorophyll content increases slightly in 90-21-3, but decreased in D937 under low potassium stress compared with the +K treatment (Table 8). The change mainly showed in the content of chlorophyll b. Overall, chlorophyll a and b in 90-21-3 increased. This observation coincided with those found in rice (Jiang and Lu, 1992). While D937 significantly decreased, chlorophyll a and b fell by 31.15 and 36.36% in 2009 respectively.

K accumulation in various organs

Analysis of variance results showed high significance between lines and K stress treatments, together with interactions among them in pairs. Figure 1 results showed that K accumulation in ach organ of 90-21-3 decreased less than D937 in two years under low potassium stress; such as potassium content of spike in 2009, 90-21-3 dropped 20.83% while D937 dropped 34.58%; Potassium content of ear, 90-21-3 dropped 41.67%, D937 dropped 59.37%; Potassium contents in stem, 90-21-3 dropped 36.56%, D937 dropped 75.63%; Potassium content in leaf declined 7.92% in 90-21-3 while declined 71.44% in D937.

K content in various leaves

Significant differences between K treatments were found for various leaves across 2009 and 2010 years. The K content of upper 8 to 1 leaf was followed by a decreasing trend under low potassium stress (Figure 2). The declines in 90-21-3 were much less than that in D937. The K centent from upper 1 to 5 leaf in 90-21-3 decreased by 10.36, 5.57, 7.14 and 10.88% in 2009, while that in D937 decreased by 51.52, 60.09, 41.26 and 24.51%, respectively. The K centents of upper 6 to 8 leaf (three ear leaves) in 90-21-3 declined significantly less than that in D937, the former decreased 21.94, 23.21 and 22.79% respectively in 2009, while they decreased in D937 by 63.41, 63.42 and 62.30%. The K content of upper 8 leaf decreased by 4.09% in 90-21-3 and 64.03% in D937 in 2009. The similar trend was exhibited in 2010.

K content in various leaf sheath

K content of various leaf sheaths declined significantly under low potassium stress in two inbred lines as shown in Figure 3. The K content in each leaf sheaths in 90-21-3 declined less than that in D937. Take ear three leaf sheaths in 2009 as an example, the declines were 21.93 to 23.21% in 90-21-3, while these were 24.51 to 63.41%

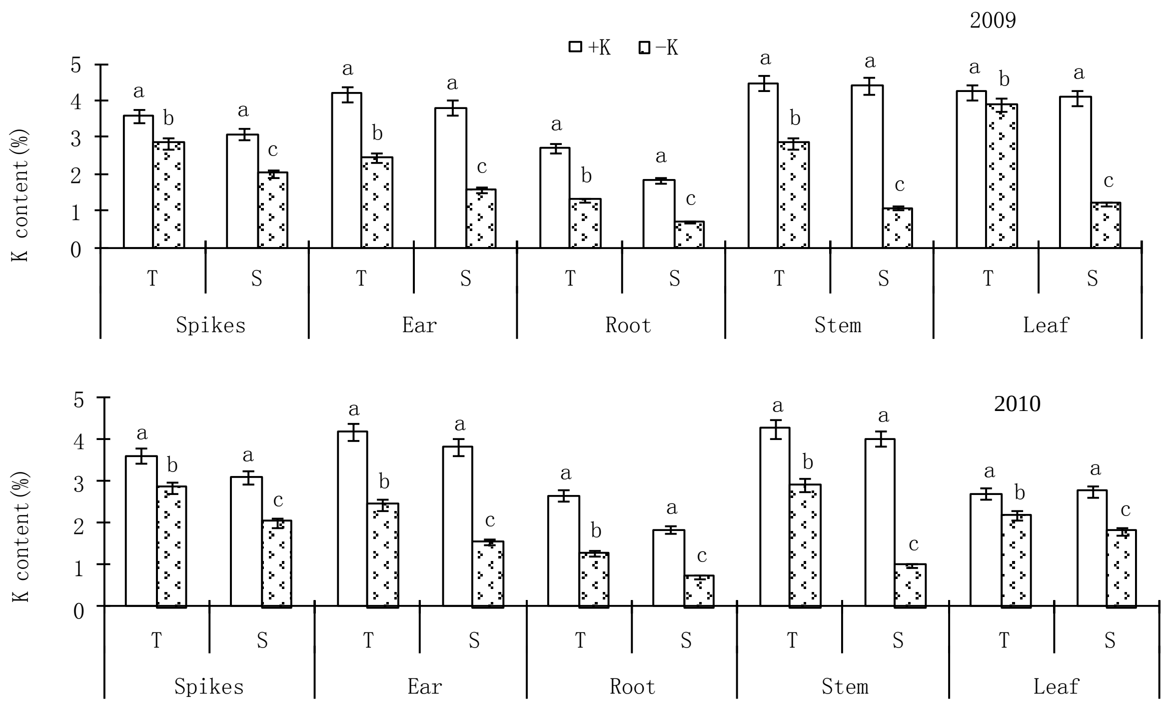

Figure 1. Effects of two K levels on potassium (K) content of each organ in 90-21-3 and D937 across 2 years. Different letters represented significant difference at 0.05 levels.

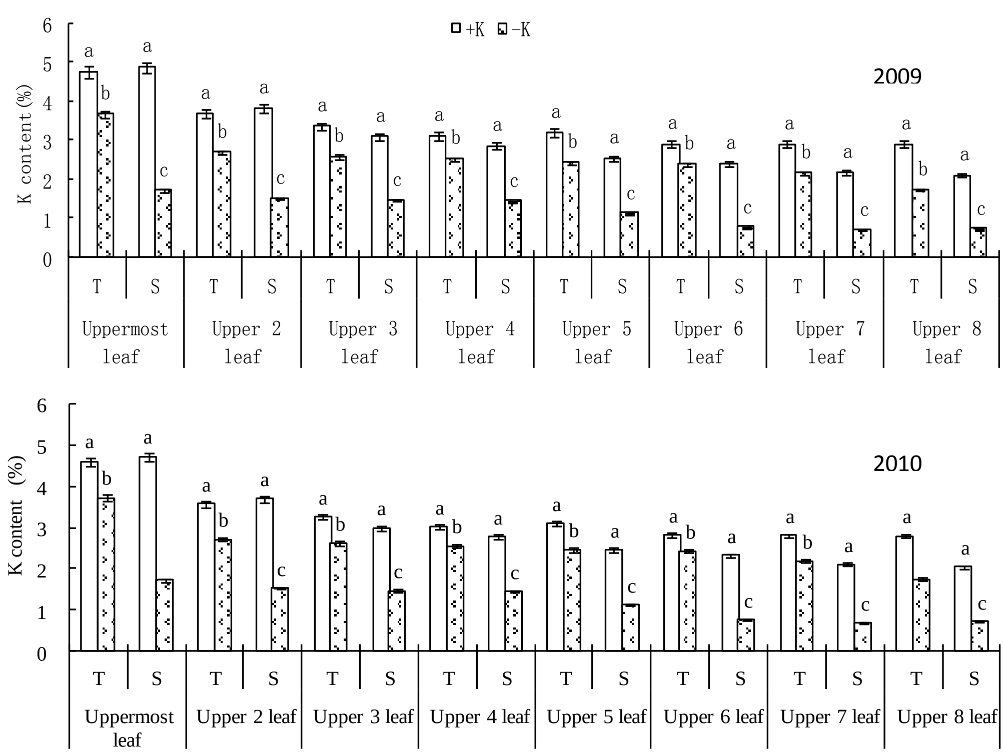

Figure 2. Effects of two K levels on potassium (K) content of each leaf in 90-21-3 and D937 across 2 years.

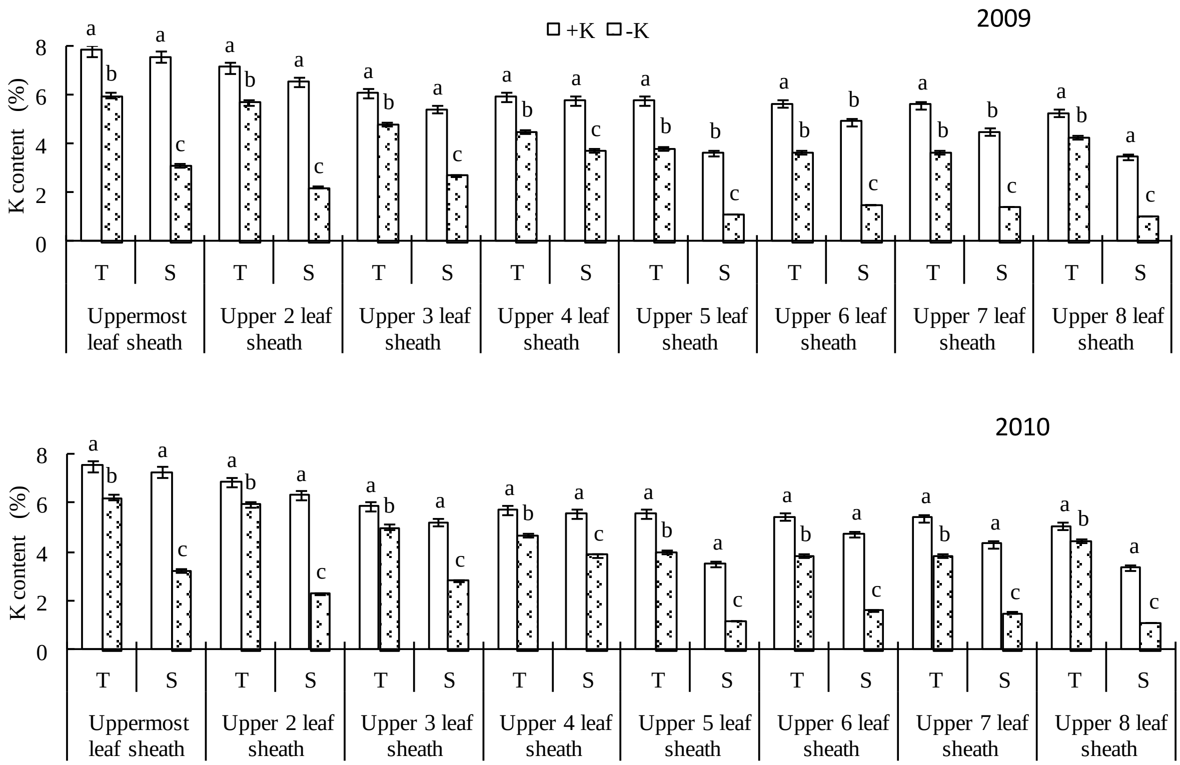

Figure 3. Effects of two K levels on potassium (K) content of each sheath in 90-21-3 and D937 across 2 years.

Table 9. Analysis of variance of the effect of inbred lines and K levels on yield and yield components across 2 years.

SOV	df	Yield per plant		No kernels of ear		1000-grain weight		Ear length		Grain number per ear	
		F.	Sig.	F.	Sig.	F.	Sig.	F.	Sig.	F.	Sig.
Year	1	1.93	0.18	0.40	0.54	0.07	0.80	1.10	0.31	4.12	0.06
K	1	357.19**	0.00	1488.40**	0.00	17.20*	0.00	3736.34**	0.00	1154.88**	0.00
Lines	1	3493.64**	0.00	1488.40**	0.00	0.27	0.61	302.52**	0.00	3850.57**	0.00
Year × K	1	0.04	0.85	0.40	0.54	0.02	0.90	17.63**	0.00	15.78**	0.00
K × lines	1	5.62*	0.03	1488.40**	0.00	7.41	0.02	1555.06**	0.00	172.98**	0.00
Year × lines	1	1.29	0.27	0.40	0.54	0.02	0.90	16.39**	0.00	23.57**	0.00
Year × K × lines	1	5.47*	0.03	0.40	0.54	1.68	0.21	12.24**	0.00	1.75	0.20

*, ** indicated the difference at 0.05, 0.01 levels, respectively.

in D937, the decrease of the 8 sheath in D937 was 62.30 and 4.08% in 90-21-3. The K centents of upper 1 to 4 leaf sheaths in 90-21-3 decreased by 10.35, 5.56, 7.13 and 10.87% in 2009, while these in D937 decreased by 51.52, 60.08, 41.26 and 24.51%.

Yield and yield components

Across 2009 and 2010 years, the results of variance analysis showed that the differences between K treatments for yield and related characters were significant (Table 9). Under low potassium stress, changes in yield and related traits were such as shown in Table 10. Yield per plant in 90-21-3 was 134.59 g under +K treatment in 2009 and 119.84 g under low potassium stress. -K stress compared with the +K treatment, it made decrease of 10.9% in 2009 and 10.4% in 2010. While the yield per plant in D937 decreased by 45.8% in 2009 and 5.7% in 2010, indicating the decline in 90-21-3 was obviously less than that in D937. Two years of yield experiments gave similar results, with a significant difference observed in the two potassium treatments; the length of no kernels, -K stress compared with the +K treatment, 90-21-3 remained 0 cm, and D937

Table 10. Effects of two K levels on yield and yield components in 90-21-3 and D937 across 2 years.

Year	Inbred lines	Treatment	Yield per plant (g)	No kernels of ear (cm)	1000-grain weight (g)	Ear length (cm)	Grain number per ear
2009	90-21-3	+K	134.59	0.00	232.70	16.77	555.00
		-K	119.84	0.00	227.52	14.53	481.00
	D937	+K	77.65	0.00	240.53	19.73	389.00
		-K	53.24	2.00	210.44	8.90	258.00
2010	90-21-3	+K	122.53	0.00	230.45	17.21	532.00
		-K	91.72	0.00	221.32	15.11	488.00
	D937	+K	74.79	0.00	240.12	18.66	411.00
		-K	55.58	2.10	210.93	9.37	285.00

Table 11. Analysis of variance of the effect of inbred lines and K levels on potassium utilization efficiency (KUE) across 2 years.

SOV	df	Straw KUE		Total plant KUE		Grain KUE	
		F.	Sig.	F.	Sig.	F.	Sig.
Year	1	1.27	0.28	1.30	0.27	0.99	0.34
K	1	1124.78**	0.00	718.49**	0.00	511.37**	0.00
Lines	1	1346.08**	0.00	1022.67**	0.00	355.69**	0.00
Year × K	1	0.46	0.51	0.27	0.61	0.07	0.79
K × lines	1	1065.20**	0.00	611.81**	0.00	92.53**	0.00
Year × lines	1	8.56*	0.01	3.87	0.07	4.61	0.05
Year × K × lines	1	0.46	0.51	0.27	0.61	0.17	0.68

*, ** indicated the difference at 0.05, 0.01 levels, respectively.

Table 12. Effects of two K levels on potassium utilization efficiency (KUE) in 90-21-3 and D937 across 2 years.

Year	Inbred line	KUE (g/mg)									RKUE
		Straw			Total plant			Grain			
		+K	-K	±%	+K	-K	±%	+K	-K	±%	-K/+K
2009	90-21-3	0.87	1.62	86.21	1.35	2.62	94.07	8.57	13.13	53.21	1.53
	D937	0.80	0.81	1.25	1.14	1.19	4.39	7.05	8.83	25.25	1.25
2010	90-21-3	0.84	1.56	82.76	1.30	2.52	90.31	8.23	12.60	51.08	1.47
	D937	0.82	0.83	1.28	1.16	1.21	4.48	7.19	9.01	25.76	1.28

increased by 2.0 and 2.1 cm in 2009 and 2010; 1000-grain weight, the decrease in 90-21-3 was 4.2% in 2009 and was 4.7% in 2010 under low potassium tress, while D937 decreased by 12.8 and 9.0% in 2009 and 2010; ear length, 90-21-3 decreased by 12.9 and 12.2% in 2009 and 2010.

The decrease in D937 were 54.9 and 49.8%; grain number, 90-21-3 decreased by 13.3 and 8.3% in 2009 and 2010, while the D937 decreased by 37.7 and 30.6%, it can be seen that the decline in 90-21-3 was far less than that in D937.

Potassium utilization efficiency (KUE)

Potassium utilization efficiency (KUE) reflects the tolerance to low potassium stress as well as whether sensitive to potassium fertilizer or not. Across 2009 and 2010 years, the KUE of straw, plant and grain showed significant difference between K treatments and inbred lines (Table 11), whereas, there was no significant difference between two years. The KUE of two maize inbred lines have been improved to some extent (Table 12). We took the average value of two years to conduct comparison. The results showed that the KUE of grain in 90-21-3 increased by 53.21%, while D937 was by 25.25%, the former was higher 2.11 times than the latter. The KUE of straw in 90-21-3 increased by 86.21%, while it increased by 1.25% in D937. The KUE of plant improved by 94.07%, D937 was by 4.39%, the former was 22.65 times more than the latter. Not only did KUE of 90-21-3 increase more than D937, but also did the relative potassium utilization efficiency (RKUE) (the ratio of the KUE in -K to +K). The RKUE of 90-21-3 was 1.53 which was higher than D937.

DISCUSSION

The important role of root growth for maintaining crop yield is becoming recognized and of increasing interest to plant breeders. With the demand for food escalating, especially in China, and expensive potash prices and K deficiency in soils became more prevalent, which is associated with maize yields, it is particularly important that we have a good understanding of the processes of K deficiency affecting root growth. Without this knowledge, it is difficult to manage soil to maximize crop production. Adequate root elongation is important for plant growth, especially in soil where resources of nutrients are scarce (Glyn et al., 2011). In this paper, K deficiency limited root elongation in D937, while the response of 90-21-3 to low potassium stress was root elongation, which would be probably the reason for its higher K absorption than D937. Deep root architecture would allow 90-21-3 to absorb more K in the soil, resulting in higher K efficiency and better growth hence higher yield. In other words, root architecture is an important agronomic trait that should not be neglected in the future breeding work. K transported readily, the research indicated that potassium content of various organs took place a slighter decline in 90-21-3 than that in D937 under low potassium stress, which exhibited soundly in three ear leaves and their sheaths. That may ensure sufficient supply of K to the ear. Accordingly, K deficiency symptoms were slighter in 90-21-3 than that in D937. As we know, typical symptoms of potassium deficiency in plants include brown scorching and curling of leaf tips as well as chlorosis (yellowing) between leaf veins. Often, potassium deficiency symptoms first appear on older (lower) leaves because potassium is a mobile nutrient, meaning that a plant can allocate potassium to younger leaves when it is K deficient.

Stay-green may be particularly advantageous for increasing crop biomass and grain yield (Duncan et al., 1981; Tao et al., 2000). Crop production depends on the level of photosynthetic duration. Therefore, keeping leaves green at the late growth stage could delay leaf senescence and extend the time of photosynthesis. The research investigated the effects of potassium deficiency on different inbred lines in filling stage. So it is of vital importance to make deep research on the effect of low potassium stress on stay-green from filling to maturity stage. We found that the number of green leaves, green leaf area per plant and the relative green leaf area of 90-21-3 declined more slowly than that of D937 under low potassium stress. It is very likely to be one of the physiological mechanisms for maize resistant to potassium deficiency. The chlorophyll content increased a little in 90-21-3, our findings were agreed substantially with those of rice (Jiang, 1996). What caused this still remains to be further studied. Whether this is due to potassium deficiency lead to block of maize growth causing the concentration effect, or to the increase of Mg^{2+} concentration promoting the synthesis of chlorophyll, further study should be conducted. If this increase were due to the former reason, the cell number or volume would give corresponding response. More experiments need to be conducted using microscope to observe the structure of cell organization. Probably, this increase may be determined by the participation of IAA. If it were due to the increase of Mg^{2+}, then what connection does Mg^{2+} have with the potassium absorption?

So far, there are 15 enzymes and 27 genes required for chlorophyll biosynthesis from glutamyl-tRNA to chlorophyll b. It was known to us that genes for all 15 steps in the pathway from glutamyl-tRNA to chlorophylls a and b have been identified in Arabidopsis (Wang, 1999). Nevertheless, in maize (*Zea mays* L.), gene location and cloning related to chlorophyll biosynthesis remains to be studied further. Keeping stay-green leaves is one of the ways in which higher dry weight can be achieved, because photosynthesis was prolonged and senescence of leaf and leaf sheath were slower in 90-21-3 than that in D937, the larger sink size get a maximum yield. Research indicated that the dry weight of 90-21-3 declined less than D937 under low potassium stress at filling to maturity stage which was mainly showed in the stalks, leaves, tassels and spikes. The more accumulation of dry matter laid the foundation for maintaining yield. Beyond that K utilization efficiency was distinct from what was discussed earlier. They were found to have increased among all inbred lines. The difference lied in 90-21-3 enhanced more prominent, its relative K efficiency was high also. That illustrated that maize inbred lines tolerant to K deficiency can absorb more potassium and effective utilization, to maintain the normal growth and metabolism of plants. We found the responses to low potassium stress of 90-21-3 mainly embodied in the root architecture of 90-21-3 was relatively strong enough to absorb K, K content of each leaf and leaf sheaths declined much smaller than D937, particularly where it found in three ear-leaves and sheaths; moreover, the maintained K contents kept the leaf stay-green in 90-21-3 were better than that in D937.

The leaf stay-green could keep longer photosynthesis prolonged. Thus, dry weight and yield were relatively higher than that of D937.

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