Effects of Potassium Deficiency on Leaf Growth, Related Water Relations and Accumulation of Solutes in Leaves of Soybean Plants

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Abstract : The influence of potassium (K) deficiency on leaf water and osmotic potentials, and concentrations of major solutes was determined to assess their contributions to the leaf growth of soybean plants grown in solution cultures containing 5.0 and 0.2 mM KCl. A deficient supply of K restricted leaf growth due to decreased mesophyll cell numbers and cell enlargement, though water content, water and osmotic potentials of the expanding leaves were not affected by the treatment. The osmolarity of the expanding leaves showed very similar values in both treatments, though the K^+ concentration in the K-deficient plants was remarkably lower than that of the control. In response to the K-deficiency, some cations, anions, sugars and amino acids accumulated in the expanding leaf, compensating the decrease in K concentration approximately 92%. These findings suggest that the leaf growth depression observed in the K-deficient plant occurred when the water relations of the K-deficient leaves are maintained close to that of the control.

Key words : Leaf growth, Leaf osmolarity, Leaf water potential, Potassium deficiency, Soybean.

カリウム欠乏がダイズの葉の生長、水分生理と溶質の蓄積におよぼす影響 : 伊藤亮一*・山岸順子・石井龍一
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要 旨 : カリウム欠乏が葉の水ポテンシャル、浸透濃度と溶質濃度に対する影響を調査し、カリウム欠乏の葉の生長に対する影響を検討した。異なる塩化カリウム濃度 (5.0 と 0.2 mM) の培養液を用いて溶液栽培したダイズ幼植物を実験に供試した。カリウム欠乏処理は葉の葉肉細胞数と細胞伸長の減少をもたらした。生長を抑制したが、伸長生長中の葉の水ポテンシャルと浸透ポテンシャル、含水率は影響を受けなかった。さらに、カリウム欠乏処理によって、伸長生長中の葉のカリウム濃度は対照区に対して顕著に減少したが、その浸透濃度は両処理間で差はなかった。そこで、カリウム欠乏に対する浸透物質濃度の変化を調査したところ、何種類かのイオン、糖、アミノ酸の濃度が対照区に比べて有意に増大し、カリウム濃度の低下の約 92% を補った。これらの結果は、カリウム欠乏によってもたらされる葉の生長の抑制は、葉の水分生理的なパラメーターはほぼ対照区と同レベルに維持されながら起きることを示唆する。

キーワード : カリウム欠乏、ダイズ、葉の浸透濃度、葉の生長、葉の水ポテンシャル。

Potassium (K) in biological systems exists always as a monovalent cation and does not participate in covalent bonding. Yet, next to nitrogen, K is the inorganic nutrient required in the largest amount by plants for metabolic functions and growth. Availability of K is known to have a large impact on crop growth. Soybean, as well as another 15 species grown at a low K level, showed less growth for dry matter production¹²⁾. In spinach seedlings supplied with various amounts of K_2SO_4 , fresh weight of the spinach tops correlated with K^+ concentration in leaves¹⁰⁾.

An important primary effect of K-deficiency is a reduction in expansive growth. When K supply was restricted, leaf growth of bean plants was suppressed due to a decrease in

pressure potential of the expanding leaves by approximately 0.2 MPa⁸⁾. The K^+ concentration in the cytoplasm is usually considerably high (ca. 100 mM) except in very deficient plants³⁾. Hence, K^+ usually accounts for an important fraction of cell osmolarity. For example, in expanding stems and leaves, K^+ has accounted for about a third of the measured osmolarity^{2,5,8)}. Because of the essential role of K in osmolarity, it has been stated that the observed lower turgor in conditions of K-deficiency is responsible for slower growth³⁾.

Some reports clearly document that the K-deficiency affects solute accumulation. An increase in some cations and anions has been observed in several species^{8,10)}. However, responses of sugars to K-deficiency showed differences across species. In soybean seedlings, K-deficient leaves had higher concentra-

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tion of sucrose and hexose sugars⁴⁾, while K-deficient leaves of bean showed a similar change in sugar concentration compared to control leaves⁸⁾. The objective of the present work was to determine effects of K-deficiency in soybean plants on (a) plant and leaf growth in relation to leaf water status; and (b) solute accumulation in leaves at different positions.

Materials and Methods

1. Plant materials and growth conditions

Seeds of soybean (*Glycine max* (L) Merrill cv. Norin 2) were germinated in moist vermiculite, and, 10 days after germination, when the primary leaves were fully expanded, uniform seedlings were transplanted to 12 L plastic containers filled with continuously aerated nutrient solutions. The seedlings for each container were held by their stems with a polystyrene board that suspended the roots in the solution. The composition of the nutrient solution was as follows: 4 mM Ca (NO₃)₂, 1 mM NH₄H₂PO₄, 2 mM MgSO₄, and other micro-constituents, such as Fe in the form of Fe-EDTA, MnCl₂, H₃BO₃, ZnSO₄ and (NH₄)₆Mo₇O₂₄. We used two treatments: One received K⁺ concentration of 5.0 mM, the other 0.2 mM. Potassium was added as KCl. Thus the two treatments differed only in K⁺ and Cl⁻ concentration. Nutrient solution was replaced every 3 days, and the pH was adjusted to 5.5. The plants were grown in a glass house (Day/night temperature, 25/20°C; Natural light).

2. Whole plant growth

Plants were harvested three times at intervals of 7 days after the 2nd trifoliolate leaves fully expanded. On harvesting days, six plants of each treatment were used for leaf area and dry weight determination.

3. Cell number

Mesophyll cell number per leaf was determined according to Brown and Rickless¹⁾. Two leaf discs (10 mm diameter) excised from subsidiary leaves of the fully expanded, 5th leaf were placed for 24 h in 5 mL of 5% chromic acid at 25°C. The softened leaf discs were then ruptured by several points using a needle and 10 mL syringe. We counted the cell number of the leaf discs under a light microscope using a hemocytometer (Erma Optical

Work, Tokyo), and calculated mesophyll cell numbers of the subsidiary leaves on the assumption that the cell number of a leaf was in proportion to the leaf area. Six samples of each treatment were used.

4. Leaf growth and leaf water relations

In order to elucidate the relationship between potassium deficiency and leaf growth rate in detail, leaf length of the central leaflets of the expanding, 5th leaves was measured daily along the midrib with a ruler. The control and K-deficient plants showed similar growth rate for leaves less than 6 cm in length. The leaf elongating rate of the K-deficient plants was lower than that of the control, and the rates were relatively stable for leaves between 7 and 9 cm in length. Hence, water and osmotic potentials of expanding leaves as selected on the basis of length, 7 to 9 cm, were measured with dew point hygrometers (C-51 sample chambers, Wescor Inc.). Leaf discs were cut between 11 a.m. and 12 p.m. and immediately transferred to the chambers enclosed with a polystyrene box. The remaining leaves used for water potential measurement were excised, wrapped in polyethylene bags, frozen on dry ice, and stored in a refrigerator at -70°C. After thawing at room temperature (ca. 25°C) for 30 min, the samples were pressed with a garlic squeezer to obtain 8 µL of sap to measure osmotic potential. Pressure potential of the leaf was calculated as the difference between water and osmotic potential. We made eight measurements for each treatment.

In another series of the experiment, the tension of xylem solution was measured with a pressure chamber. In order to minimize water loss upon sampling, leaves were covered with a plastic bag before being excised at the petiole with a razor blade and then sealed in a pressure chamber. To measure osmotic potential of apoplastic solution⁶⁾, the cut end of the petiole was washed with distilled water and blotted dry with tissue paper prior to raising the pressure in the chamber to 2.0 MPa. At this pressure, apoplastic solution was exudated at the cut surface, and 15 µL was collected in approximately 5 min. The osmotic potential of apoplast exudate was determined by thermocouple psychrometry. The water potential of the leaf apoplast solution can be estimated as the sum of the tension of the xylem solution

and the osmotic potential of the apoplastic solution⁹). Replications for each treatment were 8.

Thus, two separate methods, the pressure chamber and thermocouple psychrometry, were used to determine effects of the K-deficiency on bulk leaf water relations.

5. Osmolarity and osmotically active substances

Osmolarity and osmotically active substances were determined in two different experiments. In one experiment, the expanding, 5th leaves were used for the determination of K⁺ and osmolarity to evaluate relationships between them. In the other experiment, when the 6th trifoliolate leaves were expanding, the expanding leaves (the 6th), the fully expanded upper most leaves (the 4th) and leaves at the lower positions (the primary and the 1st) were harvested for measurement. Osmolarity and osmotically active substances of the leaves were determined as follows. The extracted leaf sap obtained by the method described above was prepared to determine osmolarity, amounts of cations and anions. The osmolarity of the sap was measured by psychrometry. The K⁺, and Mg²⁺ and Ca²⁺ were determined by flamephotometry and atomic absorption spectrometry, respectively. Anions, such as Cl⁻, NO₃⁻, SO₄⁻ and H₂PO₄⁻, were measured by ion exchange chromatography according to Kikata⁷). Sugars in the leaf were extracted in boiling water for 10 min, homogenized with a pestle and mortar. After centrifugation at 14,000 rpm for 10 min, sucrose and hexose in the supernatant were measured enzymically¹¹) with F-Kits (Boehringer Mannheim-Yamanouchi). For amino acid determination, the harvested leaves were plunged into liquid

nitrogen, and preserved at -70°C. The leaf tissues were freeze-dried, and used for extraction according to the method previously reported¹³). The analysis of amino acids was made with an amino acid auto analyzer (Shimazu, Kyoto). Concentrations of all osmotic substances were expressed on a tissue water basis. Replicate numbers for determination of osmolarity and K⁺, other cations and anions, sugars, and amino acids were 10, 6, 9 and 5, respectively.

Results

Potassium had a marked influence on plant growth (Table 1). The plants grown at the 0.2 mM K level showed significant depression in the total leaf area per plant on the 27th day after germination, and in dry weight of leaves, stems and roots as well as the total leaf area on the 34th and 41st days of the experimental period, respectively. Leaf initiation was not affected by the K-deficient treatment because by the late time of harvesting, the plants both treatments had 8th trifoliolate leaves on the main stem. Hence, the reduction in total leaf area per plant resulted from decreased area of the individual leaves.

Table 2 shows the leaf area and mesophyll cell numbers of the fully expanded subsidiary leaf of plants grown at the 0.2 and 5.0 mM K levels. The leaf area and mesophyll cell numbers were depressed 57% and 85% by the K-deficient treatment to those of the control plant, respectively. This means that the observed depression of leaf area caused by the K-deficiency was due to depression of both cell division and enlargement.

Water and osmotic potentials were measured with a thermocouple psychrometer to

Table 1. Effects of K-deficiency on the growth of soybean plants.

Days after start of germination	Treatment KCl(mM)	Leaf area (cm ²)	Dry weight(g)		
			Leaves	Stems	Roots
27	5.0	224	0.53	0.25	0.27
	0.2	181*	0.49	0.21	0.25
34	5.0	583	1.08	0.74	0.53
	0.2	397**	0.87**	0.48**	0.40**
41	5.0	1186	2.25	1.77	0.73
	0.2	741**	1.56**	0.99**	0.51**

**, * Significant differences to the control by t-test at 1% and 5% levels, respectively.

Table 2. Effects of K-deficiency on the leaf area and number of mesophyll cells in subsidiary leaflets.

Treatment KCl(mM)	Leaf area (cm ²)	Number of mesophyll cells ($\times 10^7$)
5.0	78	15
0.2	44**	12*

**, * Significant differences to the control by t-test at 1% and 5% levels, respectively.

evaluate effects of the K-deficiency on leaf growth rates, and the water relations of the expanding leaves are presented in Table 3. The leaf elongation rate was restricted by the K-deficient treatment. However, the values of water potential for the K-deficient leaves were identical to those of the control. Osmotic potential was at a slighter negative value, by 0.05 MPa in the control plants, although there was not significant difference between them. Thus, the K-deficiency did not affect the pressure potential of the expanding leaves significantly. These trends were confirmed by estimating leaf water potential in an alternative manner. Table 4 shows that values of the xylem tension were the same for the K-deficient and control plants. Osmotic potential of apoplastic sap, forced out of excised leaves under pressure, was 0.04 MPa more positive in the K deficient leaves, and calculated values

for apoplastic water potential were very similar to those determined using the thermocouple psychrometer (Table 3).

The relationship between K⁺ concentration and osmolarity of the expanding leaves showed positive significant correlation both in the control and the K-deficient plants (Fig. 1). Though the K⁺ concentration of the K-deficient leaves was remarkably lower than that of the control, the osmolarity of the both leaves showed very similar values.

Because osmolarity of the expanding leaves was not affected by the K-deficiency, it became necessary to evaluate responses of solutes to K-deficiency for further analysis. Table 5 shows leaf water content, osmolarity and some cations and anions in the expanding, fully expanded upper most and the lower leaves of the plants grown at the 0.2 and 5.0 mM K levels. Leaf water content and osmolarity in the expanding leaves showed no significant difference between the control and K-deficient plants. On the contrary, in the upper most fully expanded and lower leaves, the leaf water content decreased and osmolarity increased with the K-deficient treatment. At the 0.2 mM K level, leaf K concentrations were reduced at all positions compared to the control. To compensate for this decrease in K concentration, Mg²⁺ concentration increased in every leaf position, and Ca²⁺ concentration did so in the lower leaves, significantly. On the contrary, the anions responded to the K-

Table 3. Effects of K-deficiency on the leaf growth rate (LGR), leaf water potential (LWP), leaf osmotic potential (OP) and calculated pressure potential (PP) of the expanding leaves.

Treatment KCl(mM)	LGR (cm/d)	LWP (MPa)	OP (MPa)	PP (MPa)
5.0	1.6	-1.01	-1.18	0.19
0.2	1.1**	-1.01	-1.14	0.15

** Significant differences to the control by t-test at 1% level.

Table 4. Effects of K-deficiency on the xylem tension (XT), apoplastic osmotic potential (AOP), apoplastic water potential (AWP), leaf osmotic potential (OP) and calculated pressure potential (PP) of the expanding leaves.

Treatment KCl(mM)	XT (MPa)	AOP (MPa)	AWP (MPa)	OP (MPa)	PP (MPa)
5.0	-0.91	-0.16	-1.07	-1.18	0.11
0.2	-0.91	-0.12	-1.04	-1.14	0.10

deficient treatment differently: the Cl^- and SO_4^{2-} concentrations decreased, and NO_3^-

and H_2PO_4^- increased.

Effects of K-deficiency on leaf sugar and amino acid concentrations are presented in Tables 6 and 7, respectively. Compared to the control, the K-deficient leaves exhibited higher sugar concentrations. Glucose concentration at every leaf position and fructose concentration in the fully expanded and lower leaves increased significantly responding to K-deficiency. Amounts of total free amino acids increased by 6 mM for the expanding leaves, and 22 mM for the fully expanded most upper leaves (Table 7). Amino acids contributing strongly to the increase were asparagine and arginine. In the lower leaves, however, the K-deficiency did not affect total amino acid concentration.

Discussion

The present research clearly shows that K-deficiency depressed the number of cells and cell enlargement in leaves. It restricted leaf growth in soybean plants, though water

Fig. 1. Relationships between K concentration and osmolarity of the expanding leaves in soybean plants grown in solutions containing 5.0 (High K) or 0.2 (Low K) mM KCl. **, * Significant at 1 and 5% levels, respectively.

Table 5. Effects of K-deficiency on leaf water content (LWC), the osmolarity (Os), concentrations of some cations and anions in expanding leaves (the 6th), fully expanded most upper leaves (the 4th) and leaves at lower leaf position (the 1st).

Treatment KCl(mM)	Leaf position	LWC (%)	Os (mOs/kg)	K^+	Mg^{2+}	Ca^{2+}	Cl^- (mM)	NO_3^-	SO_4^{2-}	H_2PO_4^-
5.0	6th	80.3	409	153	18	25	9	13	21	35
	4th	79.9	410	148	35	84	17	15	23	34
	1st	80.3	402	97	58	115	29	4	20	29
0.2	6th	79.1	402	90**	37**	41	5**	25**	14**	41*
	4th	78.2*	446*	42**	78**	90	6**	19	12**	39
	1st	75.3**	546**	48**	118**	141*	11**	7	13**	48**

**, * Significant difference to the control by t-test at 1 and 5% levels, respectively.

Table 6. Effects of K-deficiency on glucose (Glu), fructose (Fru) and sucrose (Suc) concentrations. For other symbols, see Table 5.

Treatment KCl(mM)	Leaf position	Glu	Fru (mM)	Suc	Total
5.0	6th	22	23	3	48
	4th	15	17	10	42
	1st	12	18	20	51
0.2	6th	27*	28	3	58
	4th	29**	35**	10	74**
	1st	40**	67**	18	125**

**, * Significant difference to the control by t-test at 1 and 5% levels, respectively.

Table 7. Effects of K-deficiency on amino acid concentration (mM). For other symbols, see Table 5.

Treatment KCl(mM)	Leaf Position	ASP	THR	SER	ASN	GLU	GLN	GLY	ALA	VAL	MET
5.0	6th	0.60	0.11	0.69	3.26	0.55	0.32	0.42	0.44	0.12	0.03
	4th	0.50	0.10	0.66	1.30	1.62	0.31	0.28	0.19	0.05	0.02
	1st	0.16	0.05	0.28	1.00	0.70	0.32	0.11	0.03	0.05	0.04
0.2	6th	0.67	0.14*	0.84*	4.99	0.76	0.66	0.51	0.62	0.19**	0.04
	4th	0.16**	0.24	1.32**	4.39**	3.72	0.23	0.36	0.12	0.20**	0.01
	1st	0.12	0.06**	0.41*	0.69	0.62	0.34	0.16	0.16	0.06	0.01

Treatment KCl(mM)	Leaf Position	CYS	ILE	LEU	TYR	PHE	GABA	HIS	LYS	ARG	Total
5.0	6th	0.01	0.06	0.04	0.01	0.12	0.92	0.07	0.02	5.42	13.2
	4th	0.01	0.03	0.03	0.03	0.10	0.72	0.01	0.02	0.54	6.5
	1st	0.00	0.03	0.04	0.02	0.05	0.39	0.01	0.01	0.01	3.3
0.2	6th	0.01	0.09	0.07**	0.02*	0.20**	1.36*	0.10	0.04	8.02*	19.3**
	4th	0.13	0.42**	0.34**	0.40**	0.45**	0.61	0.13**	0.04**	15.11**	28.4**
	1st	0.00	0.07**	0.05	0.04**	0.04	0.31	0.02	0.02*	0.26	3.4

**, * Significant difference to the control by t-test at 1 and 5% levels, respectively.

and osmotic potentials of the expanding leaves were not affected by the K-deficiency. In this research, two separate techniques were used to measure leaf water potential, and both methods gave comparable values. These observations do not accord with the results obtained by Mengel and Arneke⁸⁾ using bean seedlings. They reported that reduction of cell enlargement was associated with an increase in the osmotic potential of the youngest leaves in inadequate K-supplied plants. All of the results obtained by the present authors indicate that analysis of osmotic substances is essential for the next step.

In order to clarify osmotic basis for effects of K-deficiency on osmolarity, osmotic substances such as some cations, anions, sugars and amino acids were determined in the expanding leaves, the fully expanded most upper leaves and the lower leaves. The K⁺ concentration decreased in the three leaf positions of the plants grown in K-deficiency, and other cations and anions, such as Mg²⁺, Ca²⁺, NO₃⁻ and H₂PO₄⁻ replaced the decrease in K insufficiently. Sugar concentrations also increased, in response to the K deficiency. Several others reported that sugar concentrations in soybean leaves⁴⁾ and spinach tops¹⁰⁾ were increased by the K-deficient treatments. In

bean seedlings, however, at the beginning of the experimental period, high sugar concentrations were observed in both the control and the K-deficient plants temporally. Later, the sugar concentrations were low and the difference between the two treatments was not observed⁸⁾.

In the present research, in addition to the sugars, the amino acids accumulated in the expanding and fully expanded leaves, and contributed to the increase in osmolarity to some extent. After all, in the expanding leaves, the decrease in K⁺ concentration induced by the K-deficiency was compensated approximately 92% through the accumulation of other osmoticum determined in this paper.

The present study clearly showed that leaf growth was restricted by the K deficiency, though water content, water potential and osmolarity of the expanding leaves were not affected. These findings may suggest a possible mechanism for leaf growth depression that the leaves can not grow adequately though the water relations of the K-deficient leaves are maintained close to that of the control.

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