



Flows of elements, ions and abscisic acid in *Ricinus communis* and site of nitrate reduction under potassium limitation

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Received 23 August 2001; Accepted 7 September 2001

Abstract

In a pot experiment *Ricinus communis* plants were cultivated in quartz sand and supplied daily with a nutrient solution which contained 4 mol m⁻³ nitrate as the nitrogen source and either full strength potassium (1.3 mol m⁻³, control) or 8% potassium (0.1 mol m⁻³, K⁺-limitation). Although the final fresh weight of the whole plant was not affected by K⁺-limitation, the root–shoot ratio was increased due to a relatively increased root growth and inhibited development of younger shoot parts. Owing to K⁺-limitation, photosynthesis was slightly decreased, while dark respiration of the shoot markedly decreased and root respiration was nearly doubled. The transport of carbon in the phloem, and to some extent in the xylem, was greater and the root was favoured in the partitioning of carbon. This was also true for nitrogen and potassium which were both taken up at lower rates, particularly potassium. In these two cases a high remobilization and recycling from the old part of the shoot was observed. By contrast, uptake of sodium was 2.4-fold higher under K⁺-limitation and this resulted in increased flows in the plants, which was discussed generally as a means for charge balance (in combination with a slight increase in uptake of magnesium and calcium). Nitrate reduction took place in the same portion in the root and shoot. This was a shift to the root compared to the control and points to an inhibition of xylem transport caused by limitation of K⁺ as an easily permeating counteranion. Low K⁺ supply also resulted in an increased biosynthesis of ABA in the roots (265%). This caused a slightly increased

deposition of ABA in the roots (193%) and a 4.6-fold higher root-to-shoot and a doubled shoot-to-root ABA signal in the xylem or phloem, respectively. The high degradation of ABA in the shoots prevented ABA accumulation there.

Key words: *Ricinus communis* L., potassium limitation, cations, anions, ABA, phloem transport, xylem transport.

Introduction

Within the last decade a series of papers has been published dealing with the effects of nutrient deficiency and nutritional disorder in long-distance solute transport and partitioning, including the stress signal abscisic acid in castor bean plants. The nutritional conditions included the type of nitrogen source (Peuke and Jeschke, 1993), salt stress (Peuke and Jeschke, 1995; Peuke *et al.*, 1996), foliar application of nitrogen (Peuke *et al.*, 1998) as well as the limitation or deficiency of nitrogen (Peuke *et al.*, 1994a) or phosphorus (Jeschke *et al.*, 1996, 1997a, b). However, the influence of potassium, the third most important nutrient, has not yet been investigated in the long-distance transport of solute and stress signals.

The importance of potassium for plant life is well documented. Potassium, a macronutrient for plants, is present in plant dry matter next to carbon, hydrogen, oxygen, and nitrogen and before sulphur and phosphorus. In commercial NPK fertilizers in agriculture, potassium is used to improve the yield. Potassium plays a role in a wide range of functions in plants: photosynthesis, enzyme activation, protein synthesis, osmotic

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potential, and as a counterion to inorganic ions and organic biopolymers (Marschner, 1995).

Whereas the mobility and long-distance transport of potassium in the xylem and phloem has been studied intensively in the past (in xylem sap, wheat: Barneix and Breteler, 1985; bean: Cakmak *et al.*, 1994a, b; *Ricinus*: Peuke and Jeschke, 1993, 1995; Peuke *et al.*, 1994a, 1998; rye: White, 1997; *Lupinus*: Jeschke *et al.*, 1985; in phloem sap, *Lupinus* and *Spartium*: Pate *et al.*, 1974; Jeschke *et al.*, 1985; *Ricinus*: Peuke and Jeschke, 1995), few data have been published about the effects of potassium supply on the transport of solutes, assimilates and hormonal stress signals, such as ABA.

The role of potassium must be viewed in conjunction with other ions, elements, and solutes. The importance of potassium for the uptake, translocation and reduction of nitrate has been shown (Blevins *et al.*, 1978; Barneix and Breteler, 1985; Förster and Jeschke, 1993; Casadesús *et al.*, 1995). The major role of potassium in this context is to act as a counterion in the xylem transport of nitrate. In bean, sucrose export from leaves was decreased by potassium deficiency (Cakmak *et al.*, 1994b).

ABA is known as a stress signal under conditions of water limitation, increased salt concentration, alkaline pH, high soil strength, and perhaps reduced nutrient supply. The effect of nutrient deficiency on long-distance ABA signals has been studied in detail (Peuke *et al.*, 1994b; Jeschke *et al.*, 1997b). The effect of N-deficiency was low compared to that of NH_4^+ given as the only N source. Further effects of N supply on phytohormone relations are cited and discussed by Peuke *et al.* (Peuke *et al.*, 1994b). Phosphorus deficiency and ABA relations have been studied earlier (Radin, 1984; Jeschke *et al.*, 1997b). These authors found a very low ABA accumulation in leaves and an increased ABA sensitivity of the stomata, despite a massively increased import of ABA in the xylem to the leaves. Only very few data have been published on the effect of potassium deficiency on ABA relations in plants. A decreased ABA accumulation has been observed in embryos of *Sorghum* and maturing seeds of *Capsicum* (Benech-Arnold *et al.*, 1995; Marrush *et al.*, 1998). By contrast, slightly increased ABA amounts in grains from potassium-deficient wheat plants have been found and an enhanced ABA amount was also found in drought-stressed and K^+ -deficient wheat flag leaves (Haeder and Beringer, 1981). The hormone contents of *Pinus silvestris* seedlings was investigated using bioassays (Michniewicz and Stopinska, 1980) and a tendency towards decreased ABA amounts in the roots of potato was observed in a bioassay study (Bhargava *et al.*, 1995).

In most of these studies, K^+ -deficiency had just minor and inconsistent effects on ABA. Transport fluids have never been analysed. It should also be mentioned that, at the time when most of these investigations were performed, only rather inefficient analytical techniques

such as bioassays were available. To complete our knowledge of the role of mineral nutrition on the long-distance transport of several solutes and on long-distance stress signals, this study has been performed with modern and sensitive analytical techniques including the investigation of long-distance transport fluids.

The first aim of the present paper is to point out the impact of limited potassium on growth, on the chemical composition of transport fluids and tissues and on the transport and partitioning of elements and ions in whole *Ricinus* plants. Additionally, the importance of potassium availability for nitrate transport in the xylem and at the site of nitrate reduction is shown. Finally, the importance of ABA as a signal for nutrient deficiency in the case of potassium is indicated.

Materials and methods

Plant cultivation

Seeds of *Ricinus communis* L. were germinated in vermiculite moistened with $0.5 \text{ mol m}^{-3} \text{ CaSO}_4$. After 13 d the plants were transferred to quartz sand culture (one plant in a 5.0 l pot) and supplied daily with nutrient solution which contained 4.0 mol m^{-3} nitrate as the nitrogen source (Peuke *et al.*, 1994a): $[\text{mmol m}^{-3}] \text{ NO}_3^- 4000, \text{ K}^+ 1332, \text{ Na}^+ 382, \text{ Mg}^{2+} 600, \text{ Ca}^{2+} 1333, \text{ Cl}^- 120, \text{ SO}_4^{2-} 610, \text{ H}_2\text{PO}_4^- 300$, and trace elements according to the Long Ashton solution (Hewitt, 1966). To induce K^+ -limitation 30 DAS, KNO_3 was reduced to 100 mmol m^{-3} and compensated for by $\text{Ca}(\text{NO}_3)_2$. The water capacity of the quartz sand was approximately 10% nutrient solution. Therefore, the daily supply was $666 \mu\text{mol}$ in the controls and $50 \mu\text{mol}$ in the K^+ -limited plants.

During growth in the greenhouse, natural light was supplemented by Osram HQL lamps (16 h light at $350\text{--}500 \mu\text{E m}^{-2} \text{ s}^{-1}$). Temperatures were between 22°C and 32°C during the day and between 15°C and 18°C at night, and relative humidity was between 50% and 70%.

Plant development and growth

During plant culture, the diameter of the hypocotyl and the length of the midrib of primary and subsequent leaves was measured.

Harvesting of plants

41 DAS, seven plants of each treatment were harvested and 10 d later a second harvest was performed. The plants were divided into roots and shoots. The shoot parts were further subdivided into old shoot (hypocotyl, first and second internodes plus the petioles and leaf laminae of leaves 1–3) and young shoot (upper internodes, leaves 4–6 and 7 at the second harvest, and the terminal bud). Every plant part was carefully washed with water or sorbitol (50 mol m^{-3}): in the case of roots to avoid leaching of solutes. Before chemical analysis, the plant tissue was immediately frozen and lyophilized. The period between 41–51 DAS represents the experimental period. During this time, xylem and phloem saps were collected at the site of the hypocotyl or third internode, and respiration and photosynthesis of plant parts were measured.

Sampling of xylem and phloem sap

Phloem sap was collected at the time of harvest (Pate *et al.*, 1974). Xylem sap was obtained as root pressure exudate at the time of harvest and additionally between the two harvesting times by applying pneumatic pressure to the root system (Passioura, 1980; Jeschke and Pate, 1991).

Measurement of respiration, partitioning of photosynthetic activity and transpiration

Root respiration of intact plants was monitored by absorbing the respiratory CO₂ in KOH in Pettenkofer vessels over the experimental time (Herridge and Pate, 1977) followed by gravimetric determination of carbonate (CO₃⁻ as BaCO₃). Dark respiration of the shoots was measured by inserting excised shoot parts into Erlenmeyer flasks of defined volume and determination of the released CO₂ by IR gas analysis (ADC, model 225 MK 3) (Layzell *et al.*, 1981). To avoid wound effects, plant parts were handled carefully. For roots, similar values were obtained with excised and with intact roots.

The partitioning of photosynthetic activity between the individual leaves was assessed on three occasions by enclosing a plant in a 30 dm³ perspex cuvette, darkening the plant for 2 min and introducing 50 µCi ¹⁴C. After thorough mixing of the atmosphere, the plants were exposed to light for 10 min (greenhouse conditions, 350–500 µE m⁻² s⁻¹) and then harvested quickly and dissected into the old and young leaves as described above. Leaf blade tissues were extracted for 6 months with 80% methanol in the cold. Soluble ¹⁴C was measured by scintillation counting and the ¹⁴C content of the plant parts was then assumed to be proportional to the contribution of each leaf to total photosynthesis (Jeschke and Pate, 1991). These data were only used to evaluate the ratio in which each plant part contributed to photosynthesis. Total net photosynthesis of the plants was obtained from the sum of all gains in carbon and of respiratory losses. Transpiration was additionally measured by porometry using a LICOR-1600 steady-state porometer.

Chemical analysis

Carbon and nitrogen in the plant tissue was determined by use of a CHN-analyser (CHN-O-RAPID Heraeus, Hanau, Germany). The element composition of the plant tissue was analysed using an ICP spectrometer (JY 70 plus, ISA, Instrument S.A. division Jobin-Yvon, France) after digestion with nitric acid under pressure for 10 h at 170 °C.

Sap was directly analysed without further extraction. Cations (K⁺, Na⁺, Ca²⁺, Mg²⁺) in the xylem sap were measured after dilution with an ionization buffer (CsCl 9.4 mol m⁻³, Sr(NO₃)₂ 57.2 mol m⁻³) by atomic absorption spectrometry (FMD 3, Carl Zeiss, Oberkochen). For anion determinations (inorganic and organic anions), xylem sap was boiled for 10 min, centrifuged and the supernatant was diluted with water before being analysed by anion chromatography with suppressed conductivity detection (Anionenchromatograph, Biotronik Co., Maintal, Germany). Within this time, less than 5% of malate degraded. Amino acids were determined using an amino acid analyser (Biotronik Co., Maintal, Germany). The amino acids were separated in this HPLC-system by ion exchange and detected after post-column derivatization with ninhydrin at 570 nm. Sucrose in the phloem sap was measured by refractometry. C/N ratios in the transport fluids were calculated from the composition of organic and nitrogenous solutes (amino acids and ammonia by an amino acid analyser and nitrate and malate by anion chromatography).

ABA analysis

Freeze-dried tissue samples were homogenized and extracted in 80% methanol. Extracts were passed through a Sep Pak C₁₈-cartridge. Methanol was removed under reduced pressure and the aqueous residue partitioned three times against ethyl acetate at pH 3.0. The ethyl acetate of the combined organic fractions was removed under reduced pressure. The residue was taken up in TBS-buffer (Tris-buffered saline: 150 mol m⁻³ NaCl 1 mol m⁻³ MgCl₂ and 50 mol m⁻³ TRIS; pH 7.8) and subjected to an immunological ABA assay (ELISA) as described earlier (Peuke *et al.*, 1994b). For phloem and xylem saps, the Sep-Pak C₁₈ purification step was omitted. The aqueous phase after partitioning against ethyl acetate was hydrolysed for 1 h at room temperature with 1 M NaOH. This fraction was acidified with concentrated hydrochloric acid to pH 3 and partitioned three times against ethyl acetate. The accuracy of the ELISA was verified for *Ricinus* in earlier investigations (Peuke *et al.*, 1994b). Recoveries of ABA during purification procedures were checked routinely using radioactive ABA and found to be more than 95%.

Modelling of flows

The flows of carbon and nitrogen were modelled according to methods described previously (Pate *et al.*, 1979; Jeschke *et al.*, 1985; Jeschke and Pate, 1991). In these methods, the data of increments in the plant organs and the concentration relations in the transport saps of elements and ions are combined on the basis of three assumptions: (1) ion uptake occurred only by the roots; (2) ions returned to the roots solely by phloem transport; (3) transport exchange took place by mass flow in the xylem or phloem. The flows of ABA were modelled according to Peuke *et al.* (Peuke, 1994b) and the flows and reduction of nitrate according to Peuke *et al.* (Peuke *et al.*, 1996).

To make the flows comparable with earlier experiments, they were calculated on the basis of the mean fresh weight (fr. wt) during the experimental period. The values of flows under potassium limitation are presented as µmol g⁻¹ fr. wt (10 d)⁻¹ and in parenthesis and italics the ratio to the control (K⁺ 1.3 mol m⁻³, see Peuke *et al.*, 1994a).

Statistics

Determinations of fresh and dry weight and ion and element content of the plant parts were obtained from seven plants for both harvests. Each plant part was analysed individually and each analysis was done with two replicates of extraction and two or three independent measurements per extraction. In the case of transport saps for the xylem 112 or 65 and for the phloem 25 or 33 saps in different fractions (site of collecting, method) were analysed for K⁺-limited or control plants, respectively. Results are given as means ± SD or SE as indicated and the comparison to the control in per cent. One way (control versus K-limitation) analysis of variance (ANOVA) was performed by the procedure GLM of SAS[®] release 8.0. Type III model sums of squares were used since the design was unbalanced.

Results

Plant growth

After the reduction of potassium supply (30 d after sowing; DAS) the secondary thickening of the hypocotyl was decelerated (Fig. 1). Additionally, the emergence and

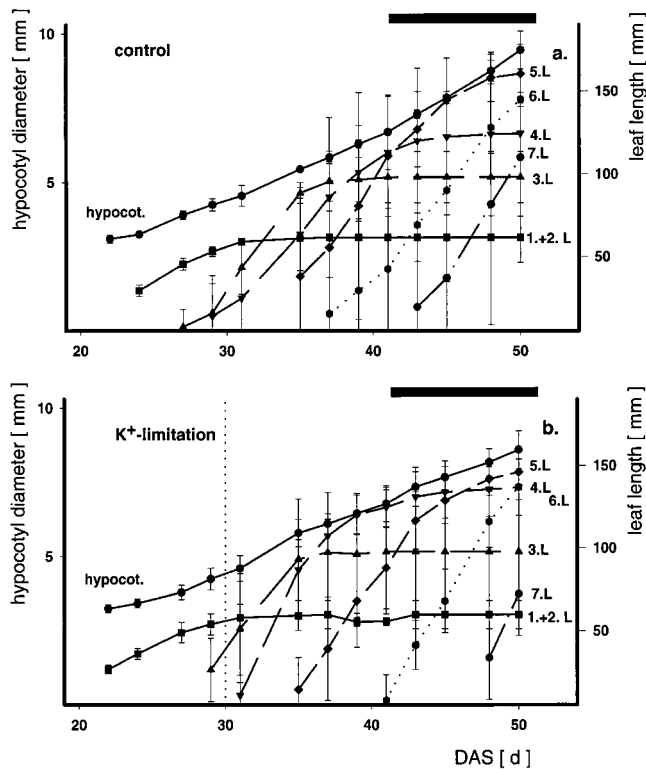


Fig. 1. Change with time of hypocotyl diameter (●), midrib length of the first and second (■), third (▲), fourth (▼), fifth (◆), sixth (●) and seventh (●) leaves of *Ricinus communis* L. The plants were cultivated in quartz sand supplied with a nutrient solution which contained 4 mol m⁻³ nitrate as the nitrogen source and (a) 1.3 mol m⁻³ potassium (control) or (b) 0.1 mol m⁻³ potassium (K⁺-limitation). The dotted vertical line indicate the time of reduction of potassium in the nutrient solution, the black box at the top of the graphs the experimental period, bars indicate standard errors of seven replicates.

growth of leaves was inhibited in response to the lower supply of potassium. While leaves 3 and 4 reached the same size as the control at the end of the experimental period, subsequent leaves remained smaller and the development of leaves 6 and 7 was delayed.

At the second harvest (51 DAS), the total biomass of the whole plant was not affected by potassium limitation, but the root–shoot ratio was strongly increased (147% of the control, Table 1). This was due to enhanced root growth (114%) and decreased shoot growth (total shoot 75%), and in particular the development of young shoot parts was inhibited. Since the development was nearly finished, the biomass of the leaves 1–3 was less affected (94%), whereas the fresh weight of the young axis and of petioles was strongly reduced (59%).

Chemical composition of xylem and phloem saps, and tissues

In xylem exudates of potassium-limited *Ricinus* plants, concentration of all inorganic ions as well as of amino acid and amide nitrogen were decreased compared to the control (Table 2). The concentration of potassium was

Table 1. Fresh weights of *Ricinus communis* at the end of the experimental period (51 DAS) in control plants and under potassium limitation

The plants were cultivated in quartz sand supplied with a nutrient solution which contained 4 mol m⁻³ nitrate as the nitrogen source and 1.3 mol m⁻³ potassium (control) or 0.1 mol m⁻³ potassium (K⁺-limitation, starting 30 DAS). Shown are means ± SD, significant differences are indicated by asterisks (***: *P* < 0.0001; **: *P* < 0.001; *: *P* < 0.05; n.s.: not significant).

	Control	K ⁺ -limitation	
Root	123 ± 4	140 ± 22	n.s.
Axis + petioles old	18 ± 2	14 ± 1	n.s.
Axis + petioles young	23 ± 2	14 ± 1	**
Leaf laminae 1–3	5 ± 1	4 ± 1	n.s.
Leaf laminae 4–7	24 ± 1	21 ± 2	n.s.
Shoot old	23 ± 2	19 ± 2	n.s.
Shoot young	47 ± 2	34 ± 2	*
Leaves	29 ± 1	25 ± 2	n.s.
Shoot	70 ± 4	53 ± 4	*
Total plant	193 ± 8	193 ± 25	n.s.
Root–shoot ratio	1.8 ± 0.2	2.6 ± 0.8	*

Table 2. Concentration of elements, ions and ABA in xylem and phloem exudates of *Ricinus communis* collected during the experimental period (41–51 DAS) under potassium limitation

The plants were cultivated in quartz sand supplied with a nutrient solution which contained 4 mol m⁻³ nitrate as the nitrogen source and 1.3 mol m⁻³ potassium (control) or 0.1 mol m⁻³ potassium (K⁺-limitation, starting 30 DAS). Shown are means ± SD and the ratio to the controls, significant differences to the controls are indicated by asterisks (***: *P* < 0.0001; **: *P* < 0.001; *: *P* < 0.05; n.s.: not significant).

	Xylem (n = 40) (mM)		Phloem (n = 12) (mM)	
K ⁺	2.7 ± 1.2***	18%	39 ± 12***	58%
Na ⁺	0.13 ± 0.06***	39%	4.6 ± 2.6***	1689%
Mg ²⁺	1.9 ± 0.6***	72%	3.2 ± 0.5 n.s.	103%
Ca ²⁺	3.7 ± 1.1 n.s.	99%	1.3 ± 0.4 n.s.	112%
NH ₄ ⁺	0.13 ± 0.03***	48%	1.83 ± 0.22 n.s.	111%
NO ₃ ⁻	12 ± 5***	45%	0.2 ± 0.7*	16%
Cl ⁻	0.7 ± 0.2***	73%	6.3 ± 1.5*	152%
Phosphate	0.4 ± 0.3***	44%	6.1 ± 1.3*	164%
Sulphate	0.4 ± 0.3***	44%	0.6 ± 0.4 n.s.	67%
Malate	n.d.		1.2 ± 1.6**	16%
Carbon	12 ± 4***	63%	553 ± 9150***	118%
Nitrogen	16 ± 6***	47%	80 ± 17.0 n.s.	84%
AA-N	4.1 ± 1.5***	55%	78 ± 17 n.s.	85%
Sucrose	n.d.		435 ± 74***	120%
	(μM)		(μM)	
ABA	0.09 ± 0.05***	241%	1.7 ± 0.9**	167%

most strongly affected and decreased to less than 20% of the control. Calcium was nearly unaffected and, under potassium limitation, it represented the major cation in xylem sap. ABA in xylem sap was more than doubled and in the phloem sap it was increased to about 170% of the control. In contrast to xylem exudates, a number of solutes were increased in phloem sap. In particular, sodium was strongly increased, its concentration being about 1700% of the control. The bivalent cations were

slightly increased as well as chloride and phosphate. Potassium and nitrogen were decreased, but not to the same degree as in the xylem exudate.

Due to potassium limitation, concentrations of potassium and total nitrogen as well as nitrate were lower in tissues of *Ricinus* plants (Table 3). This effect was more pronounced in the shoot parts than in the roots. Within the shoot, the axis and petioles showed generally stronger responses to potassium limitation. Nitrate was only found in traces in leaf laminae of K⁺-limited plants. Other inorganic ions were accumulated in dry matter due to potassium limitation. The enormous increase of sodium concentration in the roots was remarkable. Only a slight increase was observed for carbon, but, due to the high amount of carbon in the dry matter of plants, this was a big shift in total chemical composition. For ABA, contrasting observations were made: in the roots, the concentration increased slightly, while in shoot parts ABA was decreased markedly.

Flows of carbon and nitrogen

Photosynthesis, calculated from the sum of all gains in carbon and of respiratory losses, was slightly decreased by potassium limitation in *Ricinus* (92%; Fig. 2). Shoot respiration was markedly decreased (62%), whereas, by contrast, the respiration of the root was stimulated (186%). The photosynthetically gained carbon was distributed equally to root and shoot and within the shoot the young shoot received 85% of the carbon. Transport of carbon was stimulated in the phloem and also to some extent in the xylem.

The uptake of nitrogen in the form of nitrate was lowered by potassium limitation to 73% of the control (Fig. 3). 82% of nitrogen taken up was transported via

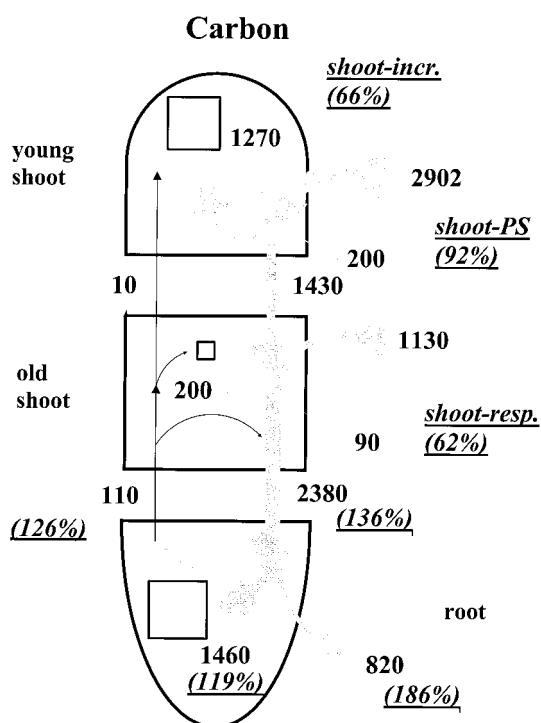


Fig. 2. Flow profiles for uptake, transport and utilization of carbon in *Ricinus communis* under potassium limitation (100 mmol m⁻³) 41–51 DAS. The shoot was subdivided into the old (leaves 1–3 plus hypocotyl and internode 1–2) and young (leaves 3–7 plus following internodes and the bud) part. The arrows on the left site (filled) indicate flow in the xylem; right (grey) flow in the phloem. The squares indicate the increment in the plant parts. The arrows leaving the plant indicate the respiration. The numbers indicate $\mu\text{mol g}^{-1}$ fr. wt (10 d)⁻¹, in parenthesis and italics the ratio to the controls (1332 mmol m⁻³ potassium) are shown. shoot-incr.: Increment in the total shoot, shoot-PS: photosynthesis in the total shoot, shoot-resp.: dark respiration of the total shoot.

Table 3. Concentration of elements, ions and ABA in tissues of *Ricinus communis* at the end of the experimental period (51 DAS) under potassium limitation

The plants were cultivated in quartz sand supplied with a nutrient solution which contained 4 mol m⁻³ nitrate as the nitrogen source and 1.3 mol m⁻³ potassium (control) or 0.1 mol m⁻³ potassium (K⁺-limitation, starting 30 DAS). Shown are means \pm SD and the ratio to the control, significant differences are indicated by asterisks (***: $P < 0.0001$; **: $P < 0.001$; *: $P < 0.05$; n.s.: not significant). Due to a different experimental design in control plants leaf laminae were analysed *in toto*.

	Roots		Axis and petioles		Leaf laminae			Shoot		Plant		
					Old	Young	Total					
	(mmol g ⁻¹ dry wt)											
C	33 \pm 0.6 n.s.	102%	34 \pm 0.7***	106%	34 \pm 0.5	36 \pm 0.4	35 \pm 0.4*	99%	35 \pm 0.4 n.s.	101%	34 \pm 0.5 n.s.	101%
N	1.4 \pm 0.1 n.s.	94%	1.0 \pm 0.1*	83%	1.9 \pm 0.2	2.8 \pm 0.5	2.6 \pm 0.4*	86%	2.0 \pm 0.2***	80%	1.7 \pm 0.2***	82%
	($\mu\text{mol g}^{-1}$ dry wt)											
Na ⁺	286 \pm 36***	204%	23 \pm 7***	264%	2.3 \pm 0.4	2.0 \pm 0.5	2.0 \pm 0.4***	81%	10 \pm 3***	225%	125 \pm 18***	245%
K ⁺	364 \pm 58***	36%	242 \pm 72***	25%	172 \pm 34	214 \pm 44	207 \pm 42***	35%	221 \pm 46***	31%	281 \pm 47***	34%
Mg ²⁺	302 \pm 39 n.s.	108%	252 \pm 33*	123%	272 \pm 22	196 \pm 46	208 \pm 40 n.s.	109%	221 \pm 33 n.s.	113%	255 \pm 34 n.s.	113%
Ca ²⁺	219 \pm 21***	134%	450 \pm 75*	88%	780 \pm 340	565 \pm 142	607 \pm 135**	154%	534 \pm 94*	123%	403 \pm 60*	118%
Cl ⁻	79 \pm 21 n.s.	165%	221 \pm 37 n.s.	131%	65 \pm 31	57 \pm 8	57 \pm 8 n.s.	116%	114 \pm 15 n.s.	126%	99 \pm 18 n.s.	131%
NO ₃ ⁻	100 \pm 25***	56%	157 \pm 87 n.s.	60%	0.1 \pm 0.2	n.d.	0.02 \pm 0.05***	0%	52 \pm 48*	49%	71 \pm 38*	54%
	(nmol g ⁻¹ dry wt)											
ABA	613 \pm 390***	131%	540 \pm 569**	35%	196 \pm 95	538 \pm 354	478 \pm 301*	22%	507 \pm 317**	26%	550 \pm 297**	37%

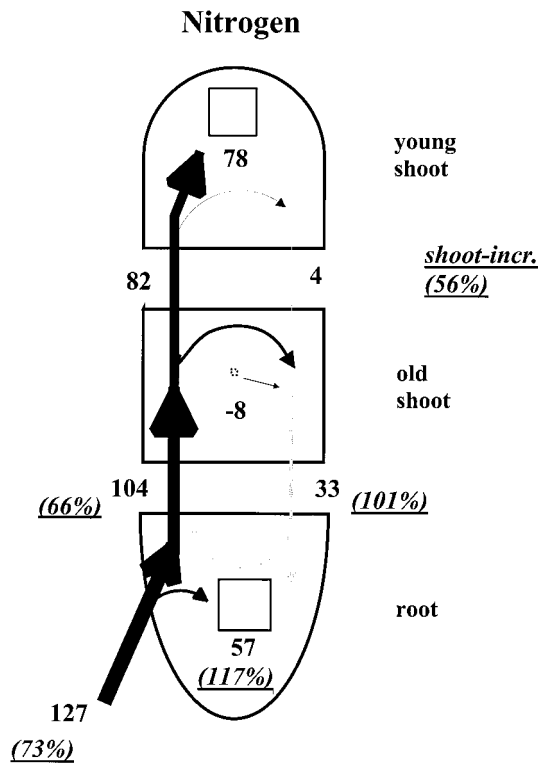


Fig. 3. Flow profiles for uptake, transport and utilization of nitrogen in *Ricinus communis* under potassium limitation (100 mmol m⁻³) 41–51 DAS. For further details see legend Fig. 2.

the xylem to the shoot, but 32% of the xylem-borne N was recycled back to the root via the phloem. The data indicate high recycling in the old part of the shoot. Although most of the nitrogen was incorporated in the shoot (55% of uptake), this was only 56% of the amount in the control. The root was favoured in the partitioning of nitrogen under potassium limitation.

Flows of cations and anions

Potassium uptake was decreased to 10% of the control because of the low supply (Fig. 4). Most of the potassium was transported to the shoot via xylem, but, due to high recycling of potassium from the shoot particularly from the old parts, a larger quantity of the potassium that was taken up was incorporated in the root. Within the shoot, potassium was incorporated only into young shoot parts. All flows of potassium were lowered, but due to the favoured role of the root, phloem flow (33%) and increment in the root (34%) were less reduced under potassium limitation than were other processes (–8% to 10% of the control).

More than 2.4-fold higher uptake of sodium was observed in K-limited plants, although the sodium supply was unaltered (Fig. 5). Most of this was incorporated in the root (96% of uptake). Although transport in the xylem and phloem was low, both of these flows were

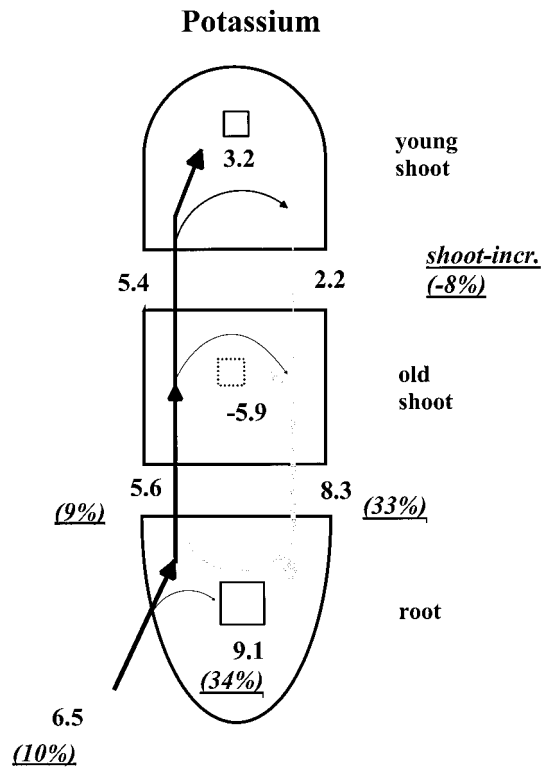


Fig. 4. Flow profiles for uptake, transport and utilization of potassium in *Ricinus communis* under potassium limitation (100 mmol m⁻³) 41–51 DAS. For further details see legend Fig. 2.

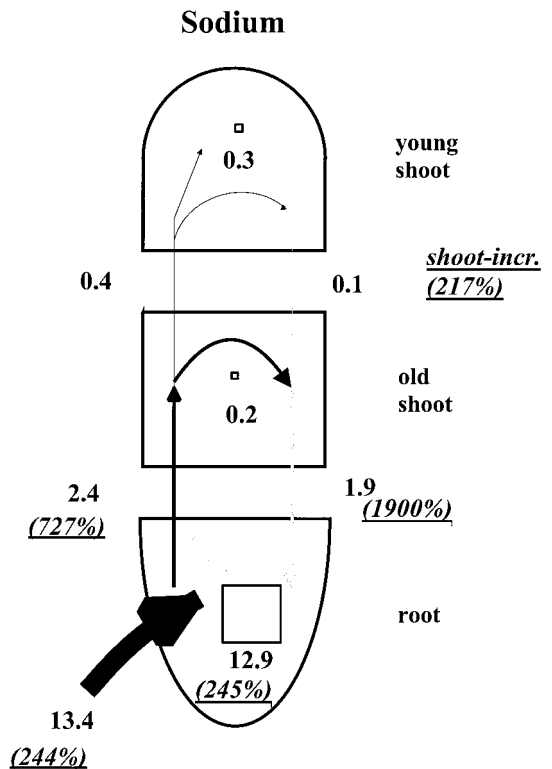


Fig. 5. Flow profiles for uptake, transport and utilization of sodium in *Ricinus communis* under potassium limitation (100 mmol m⁻³) 41–51 DAS. For further details see legend Fig. 2.

strongly increased (727% or 1900%, respectively). Sodium was also incorporated in the old part of the shoot.

The general effect of *K⁺*-limitation on the flows of magnesium, calcium and chloride was an increased uptake to about 120% of the control. As a result, flows in xylem and phloem of the plant were changed similarly and did not differ markedly from the control (data not presented).

About half of the nitrate taken up was exported via the xylem to the shoot and most of this was transported to young parts (Fig. 6). However, in relation to the control the xylem flow was lower (60%). No phloem transport of nitrate was observed. Owing to the transport behaviour, nitrate reduction took place to the same portion in root and shoot; within the shoot mostly in the younger parts. A small amount of nitrate was stored in the root.

Flows of ABA

In potassium-limited plants (Fig. 7), the synthesis (265%) as well as the increment (193%) of ABA in the roots was increased compared to the control. The strongest impact of potassium limitation was on xylem transport of ABA (463%) and phloem transport (198% of the control).

A high portion of xylem-borne ABA was recycled within the old part of the shoot and transported back to the roots via the phloem. The degradation of ABA was high in the adult and young shoots. Thus ABA did not accumulate above the levels of the controls. The leaf conductance of potassium-deficient plants seemed to be slightly decreased. However, the differences were not statistically significant, it was $782 \pm 168 \mu\text{mol m}^{-2} \text{s}^{-1}$ in the control and $640 \pm 179 \mu\text{mol m}^{-2} \text{s}^{-1}$ under *K⁺*-limitation.

Discussion

Effects of potassium limitation on growth and development

In the present study, an increase in root–shoot ratio due to *K⁺*-limitation was observed, which could be related to reduced growth of the young shoot, but there was no reduction of total biomass at the end of the experimental period. A corresponding effect on total dry weight was found previously, as well as the opposite for the partitioning between root and shoot (Cakmak *et al.*, 1994a; White, 1997). A slightly increased root growth was detected repeatedly under nutrient deficiency (Marschner, 1995). For N-deficiency, this was frequently observed and discussed in terms of efforts to reach new N-sources

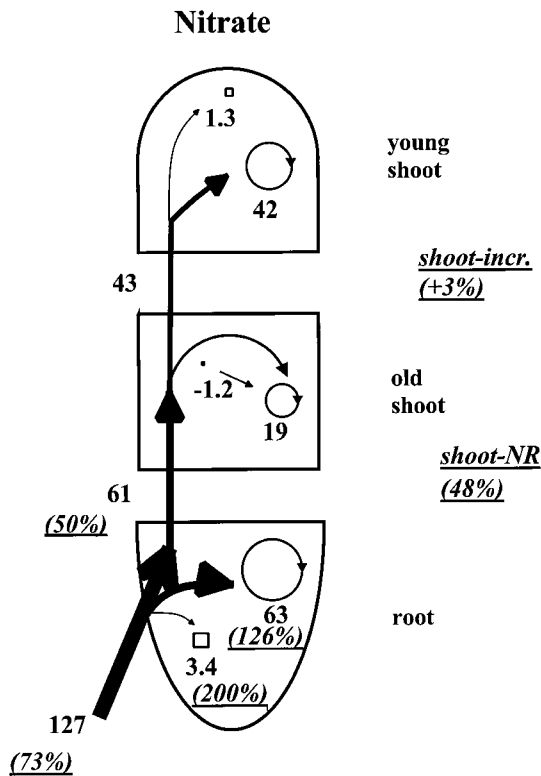


Fig. 6. Flow profiles for uptake, transport and utilization of nitrate in *Ricinus communis* under potassium limitation (100 mmol m^{-3}) 41–51 DAS. The circles indicate the nitrate reduction. For further details see legend Fig. 2.

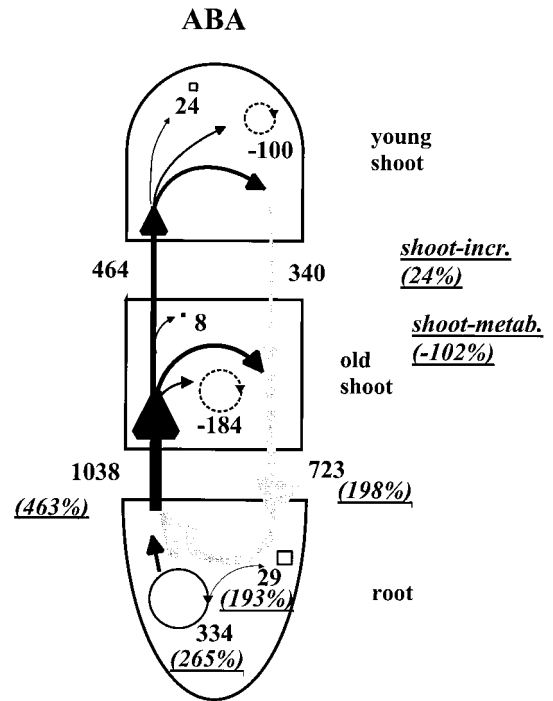


Fig. 7. Flow profiles for metabolism, transport and utilization of ABA in *Ricinus communis* under potassium limitation (100 mmol m^{-3}) 41–51 DAS. The circles indicate the net metabolism, synthesis (solid lines, positive values) or degradation (dashed lines, negative values) of ABA. For further details see legend Fig. 2.

(Rufty *et al.*, 1990). This was found earlier due to nitrogen (Peuke *et al.*, 1994a) and phosphorus deficiency (Jeschke *et al.*, 1996) in *Ricinus*. Although the concentration of potassium in the nutrient solution was reduced to only 8% of the control, the fresh weight of the whole plant was not lowered. In earlier investigations where nitrate was supplied pedospherically ($[\text{mol m}^{-3}]$: 1 N/0.33 K or 4 N/1.33 K: Peuke and Jeschke, 1993; Peuke *et al.*, 1994a) the daily K^+ -supply was taken up almost completely. This may point to a luxurious/supra-optimal use of potassium which can apparently be reduced without resulting in severe growth reduction. It can be expected, however, that reduced growth would occur over longer periods of deficiency due to the lower production of new leaves (Table 1).

Effects of potassium limitation on flows, partitioning, and concentrations of elements and ions

The observed change of growth in favour of the root was reflected in the partitioning of elements and ions. For most of the investigated elements, a higher proportion of the newly gained uptake was incorporated into the root (incorporation in the root per uptake), resulting in increased relative deposition by 6% (Mg), 8% (Ca), 14% (C), 17% (N), and 35% (Cl). Potassium itself was even exported from the shoot due to the high remobilization from the older parts of the shoot. Generally, a high recycling from xylem to phloem was observed in the old parts of the shoot. In N-limited plants, higher retranslocation of N from the shoot has been observed and even a net export of N from the shoot has been found (Pate *et al.*, 1984; Peuke *et al.*, 1994a). Additionally, the incorporation of newly absorbed N was relatively increased in the roots (Pate *et al.*, 1984; Peuke *et al.*, 1994a). Similar to these results, Cakmak *et al.* reported lowered potassium and amino acid concentration and similar concentration of magnesium in phloem exudates in bean (Cakmak *et al.*, 1994b). By contrast, sucrose export was inhibited.

The limitation of an essential nutrient in general results in decreased photosynthesis and in lowered uptake of other ions. This was true for N-deficiency in *Ricinus*, with the exception of Cl^- , for all other examined ions: K^+ , Ca^{2+} , Mg^{2+} (Peuke *et al.*, 1994a). Under P-deficiency (Jeschke *et al.*, 1996), similar to the present K^+ -limitation, photosynthesis and nitrogen uptake was inhibited. On the contrary, there seemed to be the tendency to compensate the charge balance of a missing ion by the enhanced uptake of others. This was chloride in the case of imposing low or no nitrate pedospherically (N-deficiency: Peuke *et al.*, 1994a, ammonium nutrition: Peuke and Jeschke, 1993; Peuke *et al.*, 1998). Under the present K^+ -limitation, the uptake of Ca^{2+} and Mg^{2+} were about 120% and that of Na^+ 244% of the control.

The sum of charges of these uptake rates will compensate the uptake rates of negative charges by nitrate and chloride. Generally, the missing positive charge of potassium was compensated by other cations. What was remarkable was the strong increase of sodium in the root tissue (Table 3) and in the phloem sap (Table 2). Nevertheless, compensation functions may be limited. Although, other ions can replace potassium in its vacuolar functions it cannot be compensated for in its cytoplasmic function (Marschner, 1995).

Effects of potassium limitation on the site of nitrate reduction

The site of nitrate reduction depends on plant species and environmental conditions (Pate, 1973; Andrews, 1986). Nitrate reduction was 50% in both root and shoot in K^+ -limited *Ricinus*, which represents a shift to the root compared to the control. This observation was based in part on the ratio of nitrate to total nitrogen in the xylem, which did not consider the recycling of amino acid in the phloem. As a result only half of the nitrate reduction rate of the control shoot was reached. Generally, relatively more nitrate is reduced in the roots as nitrate supply is decreased (Andrews, 1986; Rufty *et al.*, 1990; Peuke *et al.*, 1996). Although under K^+ -limitation nitrate uptake was inhibited, it was observed that at corresponding uptake rates (1 mol m^{-3} nitrate, Peuke and Jeschke, 1993) more nitrate (67%) was reduced in the shoot. As proposed before, the condition for xylem transport of nitrate plays an important role in the partitioning of nitrate reduction in the whole plant (Peuke, 2000). Under saline conditions, competition with chloride in the xylem was inhibiting nitrate transport (Peuke *et al.*, 1996). If nitrate was limiting, it was nevertheless loaded into the xylem, although it was possible to reduce the low amount totally in the root (0.2 mol m^{-3} nitrate, Peuke *et al.*, 1996). Under P-deficiency, more nitrate compared to the control was loaded into the xylem in order to compensate for the restricted availability of phosphate (Jeschke *et al.*, 1997a). In *Ricinus* and *Hordeum*, higher nitrate reduction has been found in K^+ -limited roots (Förster and Jeschke, 1993). Potassium seemed to be the favoured counter-cation for the transport of nitrate in the xylem and its limitation will consequently inhibit nitrate transport by problems with the charge balance. When KNO_3 was supplied, uptake and storage of nitrate as well as nitrate reductase activity in the leaves were increased compared to NaNO_3 as the N-source (Blevins *et al.*, 1978). Rufty *et al.* assumed that the stimulating effect of K^+ on nitrate metabolism was due to an increase of nitrate export in the xylem (Rufty *et al.*, 1981). With Ca^{2+} as the only nutrient cation, nitrate uptake was lower compared to Na^+ and K^+ as counter-cations, but, nitrate translocation in the xylem was only stimulated by K^+ in wheat (Barneix and

Breteler, 1985). Casadesús *et al.* observed a coupled loading of potassium and nitrate into the xylem (Casadesús *et al.*, 1995).

Effects of potassium limitation on ABA

The roots of castor bean responded to K⁺-limitation with an enhanced biosynthesis and deposition of ABA. This is different from earlier findings using bioassays (Michniewicz and Stopinska, 1980, 1981; Barghava *et al.*, 1995). These authors found slightly decreased ABA amounts in the roots of K⁺-deficient Scots pine and potato seedlings. Similar to conditions of phosphate deficiency a high percentage of the extra ABA was loaded into the xylem vessels and translocated to the shoot where most of it was metabolized (Jeschke *et al.*, 1997b). Thus no ABA accumulation was observed. Whereas in phosphate-deficient plants ABA acted on stomata before its degradation, no significant effect of K⁺-limitation on leaf conductance could be observed. This agrees with earlier data (Gaßner and Goeze, 1934; Ridolfi *et al.*, 1994). Earlier data on leaf ABA amounts in K⁺-deficient plants agree partly with this study's results. According to Bhargava *et al.* and Haeder and Beringer, leaf ABA of K⁺-deficient plants that are well supplied with water remained unaffected (Bhargava *et al.*, 1995; Haeder and Beringer, 1981), whereas in *Pinus sylvestris* (Michniewicz and Stopinska, 1980, 1981) and drought-stressed wheat (Haeder and Beringer, 1981) increased ABA amounts were found in leaves. Finally, the question of which factors may be responsible for stimulated ABA-biosynthesis in K⁺-deficient roots needs to be discussed, keeping in mind that quite serious water deficit or osmotic stress needs to be applied to stimulate ABA formation in roots (Hartung *et al.*, 1999). There are two possible reactions. Firstly, the increased activity of nitrate reductase may include enhanced amounts of the molybdenum cofactor (MoCo) which plays an important role in the last steps of ABA biosynthesis (Walker-Simmons *et al.*, 1989; Marin and Marion-Poll, 1997). Secondly, increased uptake of NaCl, CaCl₂ and MgCl₂ may also contribute to an increased ABA-biosynthesis (Hartung and Jeschke, 1999). Finally, a synergistic action of both factors cannot be excluded.

Conclusion

In the present study, typical symptoms of mineral nutrient limitation were found such as enhanced relative root growth, lower nutrient uptake (C, N, K⁺), and charge compensation by increased uptake of other ions (Na⁺, Mg²⁺, Ca²⁺). The applied K⁺-limitation seemed to affect growth not so strongly, although potassium supply was reduced to 8%. This points at least in part to luxurious consumption in earlier experiments and/or

effective compensation by other ions in the present experiment. In the old part of the shoot a high recycling of nutrients (N, K⁺) occurred which favoured the root in the partitioning of elements. The impact of potassium on nitrate metabolism, particularly by affecting the charge balance in xylem transport, and ABA as the root-to-shoot stress signal was demonstrated.

Acknowledgements

This paper was supported by a grant of the Sonderforschungsbereich 251 of the Deutsche Forschungsgemeinschaft to TP A3, and TP A4 and Arbeitsamt Würzburg. We thank Elfriede Reisberg, Astrid Boots and Barbara Dierich for skilful technical assistance, and Dr W Kaiser (Würzburg) for anion chromatography. The immunochemicals were generously supplied by Professor Weiler, Ruhr Universität Bochum (Germany).

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