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REGULAR ARTICLE

Shoot and root growth of hydroponic maize (*Zea mays L.*) as influenced by K deficiency

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Abstract Potassium (K) has major biophysical and biochemical functions in plant physiology. However, plant responses to K deficiency at the whole plant level are not always clearly related to these well-known functions of K at the cellular level. The objective of this study was to investigate the morphological response of maize to increasing K deficiency and test to what extent this morphological response can be interpreted in the light of the simple model proposed by Leigh and Wyn Jones, suggesting that biophysical functions are affected first. Maize was grown in a greenhouse under hydroponic conditions. For half of the plants, K was removed from the nutrient solution from the 4th visible leaf stage. The K content in the starved plants dropped from 100 to 30 mM, and was not fully compensated by an increase in other cations. Leaf elongation rates were reduced on K-deprived plants, whereas axile root elongation rates were slightly increased between 45°C days and 75°C days after starvation, and reduced thereafter. During the first part of the starvation period, i.e. under moderate K deficiency (K concentration above 40 mM), all measured variables suggest that the whole plant response may be interpreted as the consequence of the reduced leaf growth, probably due to insufficient turgor pressure or cell-wall extensibility. This general pattern of response is in agreement with the model of Leigh and Wyn Jones. However, during the second part of the starvation period, i.e. under more severe K deficiency (K concentration below 40 mM), malfunction of additional physiological processes (mostly related to biochemical functions like photosynthetic processes) must be considered to explain the plant morphological response.

Keywords *Zea mays* · Maize · Potassium · Leaf elongation rate · Root elongation rate · Root · Shoot ratio

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Introduction

Potassium (K) is the most abundant cation in plant tissues. Its roles in plant physiology can roughly be distributed between cytoplasm and vacuole, in which K is mainly present. In the cytoplasm, K plays a major role in enzyme activation (glycolysis, starch and protein synthesis, Wyn Jones et al. 1979; Evans and Wildes 1971), energetic processes (Caporn et al. 1982; Zhao et al. 2001), nutrient and assimilates transport (Hubert 1984; Pretorius et al. 1999), and the

maintenance of its concentration in this compartment is likely to be of fundamental importance. K is also suspected to influence cell-wall elasticity via its control on proton extrusion (Hsiao and Läuchli 1986; Itoh et al. 1997; Triboulot et al. 1997). In the vacuole, K can contribute to a significant proportion of cell osmotic potential, which in turn might influence the water uptake by growing cells and their pressure potential (Carroll et al. 1994). By contrast to the cytoplasmic K, the vacuolar K can be replaced by other osmotica such as Na, sugars and amino acids (Leigh and Wyn Jones 1984). Since the vacuole makes up over 90% of the cell volume in growing cells, the osmotic relations of this compartment will dominate those of the cells. All these functions (some of them interrelated) are likely to be affected during K deficiency. Two main questions thus arise, which were only partially answered by previous studies (1) is there a gradual alteration of these functions as the degree of K deficiency increases? (2) what are the consequences, on the whole plant scale, of these processes malfunctioning at the cell or tissue level? Leigh and Wyn Jones (1984) made an important contribution to the first question. They suggest that K deficiency primarily affects cell elongation physical processes (mainly via turgor reduction), whereas K-dependant enzymatic functions and other biochemical functions are maintained as long as the K concentration in plant tissue remains above a threshold value. This observation is associated with a theoretical model which assumes that, as the concentration of K in the tissue declines, the concentration in the cytoplasm is initially maintained constant, while that in the vacuole decreases. This model has since been confirmed by X-ray microanalysis, showing differential K distribution between vacuolar and cytoplasmic compartments (Leigh et al. 1986; Qi et al. 1991). Moreover, this model accounts for the possible substitution of K by other solutes (other cations and soluble sugars) for its water-related functions. Indeed such substitutions are often observed in K deficiency studies and they are likely to delay or attenuate deficiency symptoms related to its biophysical functions (Pujos and Morard 1997; Henning 2003). It must be underlined that this model is neglecting the K stored in the extracellular wall space. This view of the K functions in plant physiology therefore provides a useful interpretative framework for K deficiency studies on the whole-plant scale. However, at the plant or field scale, K deficiency symptoms are often either not linked to underlying physiological processes, or lead to divergent interpretations, especially about the K functions that might be responsible for the observed responses (El Dessougi et al. 2002; Cakmak et al. 1994; Pettigrew and Meredith 1997; Renner et al. 1995; Xi et al. 1989; Ericson 1995; Itoh et al. 1997). The lack of intermediate variable measurements, and the possible overlapping of several processes, may explain these unclear or contradictory results. The objective of this study was to investigate the morphological response of maize to increasing K deficiency and to test to what extent this morphological response can be interpreted in the light of the simple model proposed by Leigh and Wyn Jones. K deficiency effects were described at the whole plant and individual organ scales (plant biomass, leaf elongation rates, root elongation rates, etc…) and the chemical composition of organs (water, sugars, cations) was monitored to find out the underlying mechanisms associated with the observed perturbations.

Materials and methods

Plant material and experimental design

Maize seeds (*Zea mays* L. cv Cecilia) were germinated on paper towels moistened with distilled water in darkness at 28°C. After 5 days (one visible leaf stage), 93 relatively uniform seedlings were selected and each was transferred into a 28-l plastic bucket containing the following aerated standard nutrient solution (mg/l mM): KCl 135.4 *1.83*, $(NH_4)_2$ SO_4 72.6 *0.55*, $Ca(NO_3)_2$ $4H_2O$ 651.8 *2.76*, $Mg(NO_3)_2$ $6H_2O$ 87.2 *0.34*, NaH_2PO_4 6.6 *0.055*, $Na_2(HPO_4)$ $12H_2O$ 19.7 *0.055*, $MgSO_4$ 132.4 *1.1*, $MnSO_4{\cdot}H_2O$ 0.615, $ZnCl_2$ 0.21, $CuSO_4{\cdot}5H_2O$ 0.047, H_3BO_3 0.562, $(NH4)_6{\cdot}MoO_{24}{\cdot}4H_2O$ 0.322, $Fe(NH_4)_2{\cdot}(SO_4)_2{\cdot}6H_2O$·EDTA 76.4 *0.1*. The pH was adjusted to 5.5–6.5. The nutrient solution was partly renewed every day so that the K concentration never dropped below 90% of the target value. The containers were placed in a greenhouse (Bordeaux, France) under natural light.

When plants reached the 4th visible leaf stage (180 degree-days, base 10°C), 43 plants were deprived of K (treatment K0). The complete nutrient solution was replaced by a nutrient solution without KCl, which

means that the following interpretation of the results theoretically includes the absence of both ions. 43 other plants were grown on the complete nutrient solution as described before (treatment K+). Seven plants were harvested for destructive measurements (see below). The experimental design was completely randomized. The experiment lasted 23 days after starvation, until the 9–10th visible leaf stage.

Plant sampling and measurements

Eight plants per treatment were randomly chosen for daily measurements on shoots and roots. The number of visible and expanded leaves and the rank of the upper phytomer carrying emerged axile roots were recorded daily (see Mollier and Pellerin 1999, for nomenclature). For leaves 4–8, individual leaf elongation rates were recorded daily by drawing an ink mark on the growing leaves (visible, non ligulated leaves) at the height of a thin horizontal fixed twine. The elongation of one axile root per phytomer was also recorded daily by carefully removing the root system from the nutrient solution. The length of the apical unbranched zone of the root (L_{unbr} in mm) was measured, and a thin ink mark was drawn at the boundary between the branched zone and the apical unbranched zone, making possible further calculations on axile root elongation rates and first-order lateral root ages (see later). This method was previously used by Mollier and Pellerin (1999) and no different pattern was observed between marked and unmarked roots.

Parallel to this continuous non-destructive daily recording of plant stage and leaf and root elongation, seven plants per treatment were randomly sampled twice a week (about every 30 degree-days) for additional destructive measurements on roots and shoots. One sampling date occurred just before the starvation and 6 further sampling dates occurred on the K differentiated treatments. The fresh weight of roots and shoots were recorded and the following measurements were made: number of visible and expanded leaves, length (L, in m) and width (W, in m) of individual leaves, number of emerged axil roots per phytomer, length of one axile root per phytomer and diameter of its apical zone (10 mm behind the apex). A small leaf disk (50 mm^2) was sampled on the upper ligulated leaf and immediately frozen (−30°C) for further analysis of sugar content. The apical zone (10 mm behind the apex) of each measured axile root was also sampled and frozen (−30°C) for further analysis of sugar content (Muller et al. 1998). Roots and shoots were then dried at 105°C for dry weight measurement, water content calculation, and chemical analysis (see later). Additional measurements were made on plants of the last sample, (group of plants measured daily as described before): Distances between successive lines drawn on the leaves were measured and used to calculate the daily leaf elongation rates. Similarly, distances between the successive marks on axile roots were measured and used for calculating the root elongation rates (see later). The number, length and diameter of first-order laterals borne by the axile roots, and the diameter of the axile root, were measured on 20 mm segments centered on the successive ink marks so that the age of the measured laterals, i.e. the time elapsed between their emergence and the observation date, could be easily calculated. However, measurements on first-order laterals were only made on the seven youngest axile root segments carrying laterals, since older laterals were hardly measurable. Only five plants out of eight were used for these measurements, because of broken axile roots.

Biochemical analysis

K, Ca, Mg and Na were extracted from plant material in the following way: dried material was milled to pass a 1-mm sieve. This was calcinated at 550°C. Ashes were then digested in a 1-M nitric acid which was boiled off. The residue was taken up in hot 0.5 M nitric acid and the volume made up on cooling. Cations were determined by atomic emission or absorption. Their concentrations in plant tissue were then calculated relative to both dry matter (in g g^{-1}) and tissue water content (fresh minus dry weight, in mM). This last unit may be more relevant, especially for K whose main function is to generate osmotic potential through its concentration in cell vacuoles (Leigh 1989; Springob et al. 1995).

Soluble sugars (glucose, fructose and sucrose) from leaf disks and root apices were extracted in boiling 80% ethanol for 15 min. The extraction was repeated once in ethanol and a third time with water. Leaf disk samples were then transferred to an autoclave to convert starch into glucose. All extracts were evaporated to dryness and frozen until analysis (Muller et al. 1998). Soluble sugar concentrations were then measured by the enzymatic and spectroscopic method as proposed by Kunst et al. (1984).

Control of environmental conditions

The incident photosynthetically active radiation (PAR) was measured in the greenhouse using three amorphous silicon cells (Solems, Palaiseau, France) as proposed by Chartier et al. (1989). Air relative humidity was measured with a relative humidity probe (HMP35AC, Vaisala, Helsinki, Finland). Air temperature was measured using temperature probes (107, Campbell scientific, UK). All sensors were connected to a datalogger (21X, Campbell Scientific, UK). Measurements were made every 15 min, but only hourly average values were recorded. Thermal time (TT in °C days) was calculated on a daily basis as follows:

$$TT = (T_{max} + T_{min})/2 - T_b$$

where T_{max} and T_{min} are the maximum and minimum hourly air temperature (in °C), respectively, and T_b the base temperature (10°C). Cumulative thermal time was calculated by adding daily values.

Average daily values of air temperature, incident PAR and air relative humidity during the experiment were 18.6°C, 8.3 MJ m^{-2} and 71%, respectively. The average daily incident PAR was relatively low since the experiment was carried out in October without additional artificial light.

Calculations

Leaf area

Areas (A, in m^2) of individual leaves were calculated as followed (Bonhomme et al. 1982):

$A = 0.75 \times L \times W$ for fully expanded (ligulated) leaves
$A = 0.5 \times L \times W$ for expanding leaves

The total leaf area per plant (LA in m^2) was calculated by adding all individual leaf areas.

Photosynthetically active radiation absorbed by plants

The daily amount of PAR absorbed by plants (PARa, in MJ) was calculated as the product of the daily incident PAR (in MJ m^{-2}) and the total leaf area per plant (in m^2). Leaf absorbance is supposed to be equal to 1. Self-shading and mutual shading between plants were neglected because of the low total area per plant and the large distance between plants (approximately 1 plant m^{-2}). For daily calculations the leaf area per plant was linearly interpolated between sampling dates.

Radiation use efficiency

The radiation use efficiency (RUE, in g MJ^{-1}) was calculated for each interval between sampling dates as follows:

$$RUE = (W_n - W_{n-1})/(cPARa_n - cPARa_{n-1})$$

with W_n and W_{n-1} being the average plant dry biomass (in g) on dates n and $n-1$, respectively, and $cPARa_n$ and $cPARa_{n-1}$ being the cumulated amount of PAR absorbed by the plant (MJ) on dates n and $n-1$ respectively. Since RUE was calculated from average plant biomass and absorbed PAR increments at the plant population level no replications were available and no statistical tests could be performed on this variable.

Leaf elongation rate (LER)

The general shape of the curve of a maize leaf blade length against thermal time from its initiation is sigmoid (Arkebauer and Norman 1995; Durand et al. 1995). The elongation rate gradually increases, levels out (quasi-linear elongation phase) and then gradually decreases. The first phase of elongation occurs when the leaf is hidden by the sheaths of the lower leaves. To make the comparison easier between LER of different leaf ranks and K treatments, we calculated the LER during the quasi-linear elongation phase only. Only data when the leaf was between 20% and 90% of its final leaf length were considered for LER calculations. For each leaf rank and K treatment the mean LER (in mm °C $days^{-1}$) was calculated as the slope of leaf length against cumulative thermal time.

Axile root elongation rate (RER)

The root elongation rate (RER in mm °C $days^{-1}$) of axile roots was calculated using the successive ink marks as proposed by Mollier and Pellerin (1999):

$$RER = (\Delta L_{br} + L_{unbr\,n} - L_{unbr\,n-1})/(TT_n - TT_{n-1})$$

with ΔL_{br} being the distance (in mm) between the ink marks drawn on the root on days n and $n-1$, $L_{unbr\ n}$

and $L_{\text{unbr}\ n-1}$ being the length of the apical unbranched zone on days n and $n-1$ and TT_n and TT_{n-1} being the cumulated thermal time on days n and $n-1$.

Statistical tests

For most of the measured variables, analysis of variance was carried out for each day with K treatment being the only factor. Means were separated using the Least Squares Means Difference Student's Test at the $P=0.05$ and 0.01 significance levels. Statistical tests were performed using the JMP® V5 software.

Results

Plant chemical composition and water content

K concentration, expressed on a tissue water basis, was close to 100 mM during the whole experiment for K+ plants, whereas it declined steadily and dropped to about 30 mM at the end of the experiment for K-deprived plants (Fig. 1). K concentrations were significantly lower in K-deprived plants from the first sampling date after K deprivation (27.6°C days) till the end of the experiment. K concentrations were 10–15 mM lower in roots than in shoots and this discrepancy was more pronounced for K-deprived plants (data not shown). When expressed on a dry matter basis the K concentration in plants was about 0.06 g g^{-1} in K+ plants whereas it dropped to about 0.01 g g^{-1} in K0 plants at the end of the experiment. The water content in plant tissues was significantly lower for K-deprived plants from the third sampling date after K deprivation (97.4°C days), in shoots but not in roots (data not shown). The relative value of shoot water content (shoot water content in K0/shoot water content in K+) declined steadily and reached 0.95 at the end of the experiment. Therefore, the difference between K treatments in K concentrations was more pronounced when expressed on a dry matter basis (K concentration in plants of K0 plants/K concentration in plants of K+ plants=0.25 and 0.17 at the end of the experiment when expressed on a tissue water and a dry matter basis, respectively). Ca, Mg and Na concentrations were significantly higher in K-deprived plants (data not shown). This increase was in part due to a net increase in their uptake, and thus deposition rate (data not shown). But the calculated sum of cations concentration expressed towards tissue water (K, Ca, Mg and Na, added under the assumption that all these cations were under a soluble form) was however significantly lower in K deprived plants (Fig. 1). This suggests that the higher uptake of other cations (Ca, Mg, Na) by K deprived plants did not fully compensate the reduced molarity due to the lower K content.

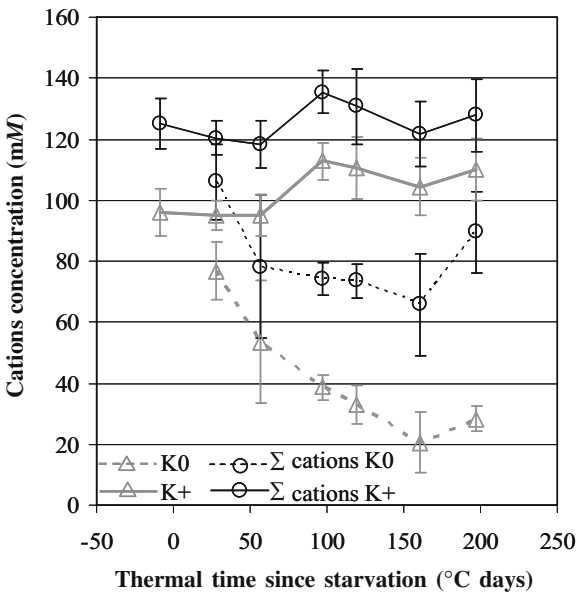


Fig. 1 K concentration and total concentration in cations (K+Ca+Mg+Na) in plant tissue water (in mM) as a function of thermal time after starvation, for treatments K0 and K+ (means±standard deviation)

Plant stages, biomass accumulation and radiation use efficiency

The number of visible leaves, the number of expanded leaves and the number of phytomers carrying axile roots were slightly lower for K0 plants at the end of the experiment (−0.5 leaves and −0.5 phytomer carrying axile roots). The differences between K treatments were statistically significant at the last sampling date only. The dry matter accumulation in shoots and roots was significantly reduced by K deprivation from the fourth sampling date (119°C days) after deprivation (Fig. 2). At the end of the experiment, the relative plant dry biomass (plant dry biomass in K0/plant dry biomass in K+) was 0.57.

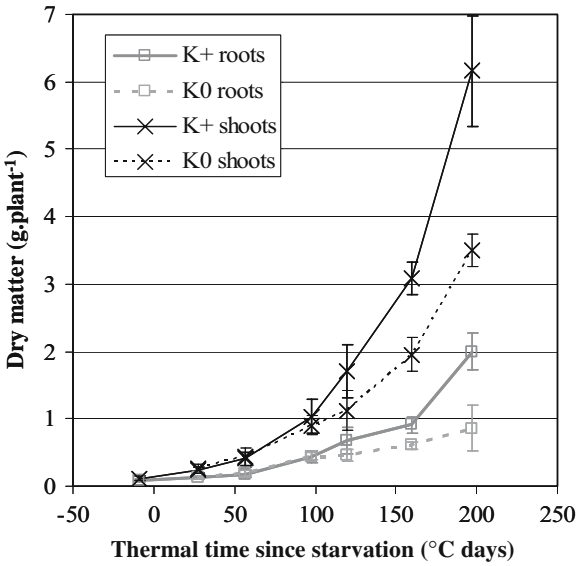


Fig. 2 Roots and shoots dry matter (in g per plant) as a function of thermal time after starvation for treatments K0 and K+ (means±standard deviation)

Shoots and roots were affected similarly, so that the root biomass/plant biomass ratio was not strongly altered. It was however slightly higher for K0 plants just after K deprivation (between the 5th and 7th visible leaf stages) and became slightly lower later on (Fig. 3). If compared day by day, the root biomass/plant biomass ratio was significantly higher for the K-deprived plants at the third sampling date (97.4°C days) and significantly lower at the sixth sampling date (196.8°C days). Although no statistical tests could be performed, the calculated values of RUE were very similar for K0 and K+ treatments for the two calculation periods just after K starvation, whereas they differed strongly later on (Fig. 4).

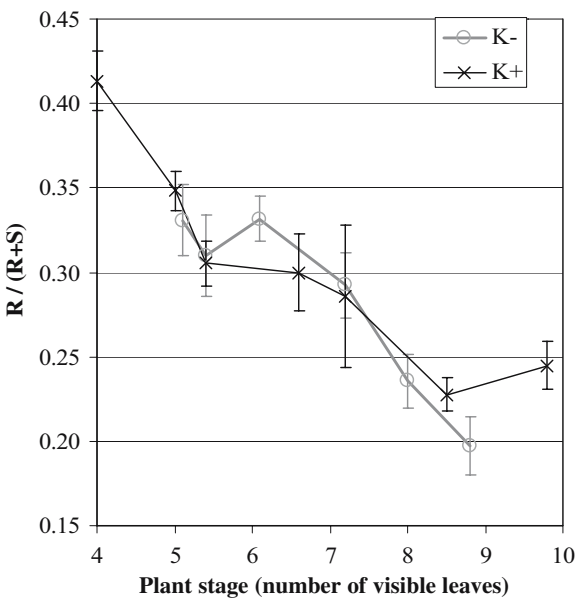


Fig. 3 Ratio between the root dry biomass (R) and the total plant dry biomass ($R+S$), as a function of the plant stage (number of visible leaves) for treatments K0 and K+ (means± standard deviation)

Leaf elongation rates

When expressed on a daily basis (in mm per day) the leaf elongation rates were linearly related to air temperatures ($R^2>0.8$, data not shown). This result confirms that leaf elongation is strongly controlled by temperature and emphasises the value of expressing leaf elongation rates on a thermal time basis (Ben-Haj-Salah and Tardieu 1995). Significant correlations were also observed between LER expressed on a thermal time basis and the incident PAR accumulated during the 3 days preceding the leaf elongation measurement ($0.34<R<0.96$, depending on the leaf rank and K treatment). This suggests that under our experimental conditions leaf elongation was also controlled by the incident light. Table 1 shows the average LER calculated during the quasi-linear elongation phase for each leaf number and K treatment and Fig. 5 shows the relative values of the

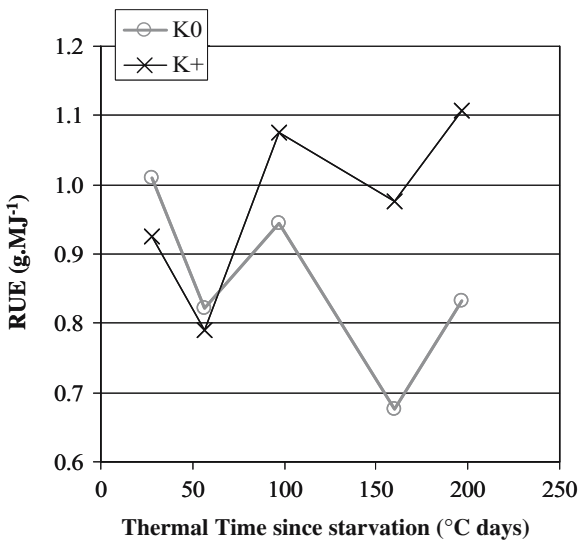


Fig. 4 Calculated values of the radiation use efficiency (in g MJ^{-1}) as a function of thermal time after starvation for treatments K0 and K+. RUE was calculated from average plant biomass and absorbed PAR increments at the plant population level

Table 1 Values of leaves elongation rates (mm °C days^{-1}) of each leaf rank for both treatments

Leaf rank	K0 treatment	K+ treatment
	LER (mm)	LER (mm)
4 n.s.	0.49±0.02	0.51±0.06
5**	0.53±0.03	0.61±0.04
6**	0.6±0.03	0.73±0.03
7**	0.58±0.04	0.76±0.02
8**	0.54±0.02	0.78±0.04

* and ** indicate significance at $p<0.05$ and 0.01 respectively. Data represent a mean of eight plants per K treatment±SD.

LER (LER for K0/LER for K+) versus thermal time after starvation for leaves 4 to 8. Average LER during the quasi-linear elongation phase were significantly lower in the K0 treatment for leaves 5 to 8 (Table 1). The relative LER decreased steadily from K starvation until the end of the experiment, with a final value close to 0.7. Values corresponding to the successive leaves partially overlapped, which suggests that two successive leaves elongating simultaneously were similarly affected by K deficiency (Fig. 5).

Axile root elongation rates and branching

The final number of axile roots per phytomer was not affected by K treatments. As for leaves, the axile root elongation rates expressed on a daily basis (in mm per day) were linearly related to temperature. When expressed on a thermal time basis, the root elongation rates were slightly correlated with the cumulative amount of incident light during the three preceding days ($0.38<R<0.74$, see Fig. 6 which shows the parallelism between the RER of axile roots from phytomer 2 and the incident light). Figure 7 shows the relative RER (RER for K0/RER for K+) versus thermal time after starvation for axile roots from phytomer 2 to 4. The relative RER was below 1 just after K deprivation, then it increased and reached 1.4 about 50 degree-days after K starvation and then decreased steadily until 0.8–0.9 at the end of the experiment. RER were significantly higher for K0 plants between 45°C days and 75°C days after starvation. As for leaves, the relative RER overlapped for axile roots of successive phytomers which suggests that axile roots elongating simultaneously were similarly affected by K treatments irrespective of their phytomer. Because of these time-dependant and contrasting effects of K treatments on RER, the total length of axile roots was never significantly different between treatments. At the end of the experiment the total length of axile roots was slightly, but not significantly, lower for K0 plants: 761 and 743, 714 and 706, 472 and 445 mm for axile roots of K+ and K0 treatments carried by phytomer 2, 3 and 4, respectively. Apical diameters of axile roots were

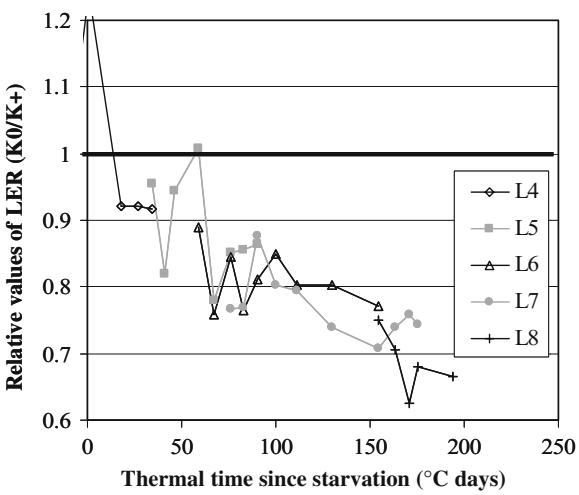


Fig. 5 Relative value of the leaf elongation rate (LER in K0/LER in K+), for leaves 4 to 8, as a function of thermal time after starvation

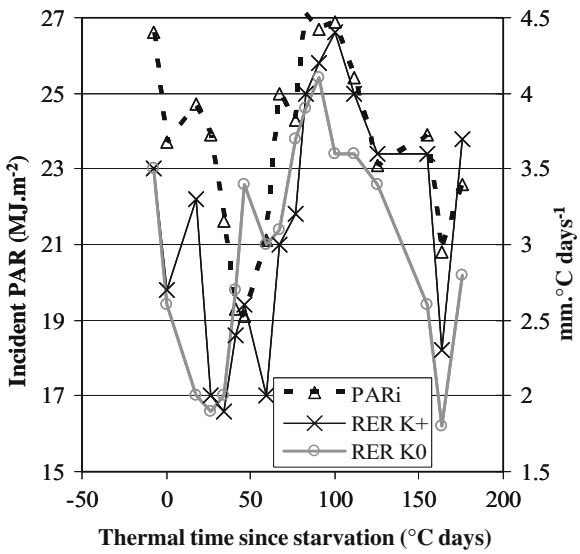


Fig. 6 Root elongation rate (in mm °C days^{-1}) of axile roots from phytomer 2 and daily incident photosynthetically active radiation (in MJ m^{-2}) versus thermal time after starvation

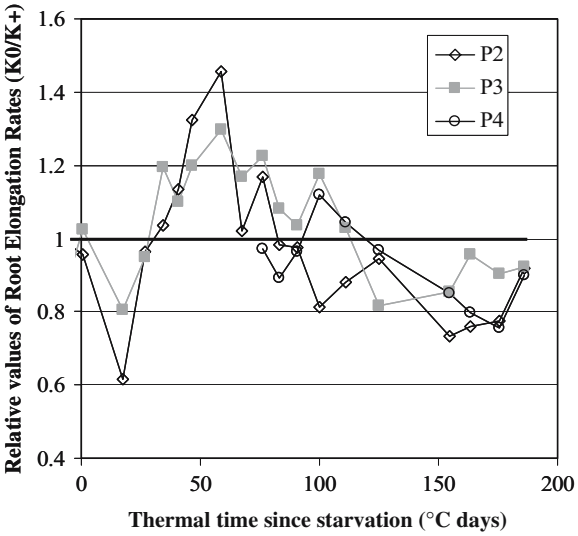


Fig. 7 Relative value of the root elongation rate (RER in K0/RER in K+), for axile roots from phytomers P2, P3 and P4, as a function of thermal time after starvation

significantly lower for K0 plants at the end of the experiment: 1.36 and 1.14 mm for axile roots of phytomer 3, 1.72 and 1.34 mm for axile roots of phytomer 4, for treatments K+ and K0, respectively.

The delay between lateral root initiation and emergence was calculated as the slope of the relationship between the length of the apical unbranched zone (L_{unbr}) and the axile root elongation rate (RER), as proposed by Pellerin and Tabourel (1995). It was not significantly different between K treatments (54 degree-days for both treatments). The density of branching (number of first-order lateral roots per unit length of axile root) was slightly higher for K0 plants, but the length of laterals of similar age were generally significantly lower (see Table 2 for phytomer 3; the same trends were observed on other phytomers). However, as mentioned previously, measurements on laterals were only possible on those which had emerged during the last 10 days of the experiment, so that no data were available on laterals whose growing period occurred just after K starvation.

Soluble sugar and starch concentrations in leaves and root apices

The concentrations of soluble sugars measured in the root apical zones were generally greater for the K0 treatment (Table 3), although the difference was not always significant. Glucose was the most abundant sugar (>70%), followed by fructose. No significant difference was observed between K treatments in the soluble sugar and starch content in mature leaves.

Discussion

K concentrations observed in plants of the K+ treatment compare well with, but are in the lower range of values reported by other authors for plants well supplied with K. Classical values reported in the literature range between 150–250 mM (40–60 mg K g^{-1} dry matter) (Koch and Estes 1975; Huber 1985; Leigh 1989; Barraclough and Leigh 1993). Values observed in K-deprived plants at the end of the experiment are close to or slightly below the critical values reported in the literature for maximum growth

Table 2 Density, length and diameter of first-order laterals on axile roots of phytomer 3, for 20 mm axile root segments observed at the last sampling date

TT since starvation	Age of laterals	Density K0	Density K+	Length K0	Length K+	Diameter K0	Diameter K+
°C days	°C days	(cm^{-1})	(cm^{-1})	(mm)	(mm)	(mm)	(mm)
185.6	10.6	14.6±2.2*	9.3±2.4	3.50±0.7*	4.90±0.7	0.34±0.05	0.39±0.1
175.1	21.1	16.4±3.9	10.8±3	5.80±1.1*	8.72±1.2	0.31±0.02	0.5±0
162.7	33.5	9.5±4.3	8.8±2.5	12.52±4.2	23.52±8.6	0.3±0.04**	0.41±0.03
124.7	71.5	11.1±2.5	10.9±3.0	12.56±3.2**	32.11±8.1	0.28±0.03	0.31±0.05
110.6	85.6	11.1±2.1	13.2±3.9	12.73±2.2**	30.67±4.5	0.3±0	0.3±0.03
99.4	96.8	11.3±0.3*	7.9±1.5	14.04±2.4*	38.49±7.6	0.28±0.02	0.35±0.06

The first column states the thermal time since starvation when lateral emerged on the root segment, whereas the second column states for the age of laterals when measured. * and ** indicate significance at $p<0.05$ and 0.01 respectively. Data represent a mean of five plants per K treatment±SD.

Table 3 Soluble sugar content in roots apex and leaf disks, and starch in leaf disks, for both treatments

TT since starvation °C days B10	Soluble sugars in roots		Soluble sugars in leaves		Starch in leaves	
	g 100 g dry matter^{-1}		g 100 g dry matter^{-1}		g 100 g dry matter^{-1}	
	K0	K+	K0	K+	K0	K+
−9.0		7.3±0.9		6.8±1.7		0.4±0.1
27.6	20.1±5.0	15.3±3.1	8.0±5.0	11.8±10	2.2±0.5	2.3±0.7
56.4	18.4±0.3**	13.5±0.9	6.0±4.1	7.7±2.1	1.5±0.6	1.1±0
97.4	17.4±2.8	14.1±1.6	4.3±1.9	5.4±1.9	0.6±0.5	0.6±0.7
119.2	21.0±2.1*	14.4±3.0				
160.1	17.4±3.3	11.0±3.0	4.8±1.0	5.5±1.2	0.2±0.1	0.5±0.3
196.8	15.7±1.1	14.4±0.5	5.5±1.3*	2.5±0.3	0.7±0.4	0.2±0.1

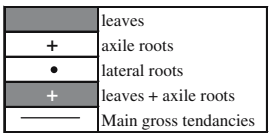
* and ** indicate significance at $p<0.05$ and 0.01 respectively. Data represent a mean of five plants per K treatment±SD.

(Peaslee and Moss 1966; Leigh and Wyn Jones 1984; Bednarz and Oosterhuis 1999; Schneider et al. 2003). The concentration of Ca, Mg and Na was increased in K-deprived plants, which is commonly observed under K deficiency (Pujos and Morard 1997; Henning 2003). However, in terms of molarity, and thus osmolarity, the higher concentrations of other cations did not fully compensate for the decrease of K concentration. It must be underlined that, unlike K, some of the Ca and Mg found in plant tissue is located outside the water-soluble portions of the plant cell. Accordingly, our procedure of using the total tissue concentrations of these cations probably overestimated the solute contribution of each to leaf osmotic potential, and then reinforce our conclusion that their contribution in the osmotic potential must have been lowered by the deficiency. Even if the contribution of the additional soluble sugars of the K-treatment plants are taken into account in the molarity of the plant water tissue, their increase wasn't high enough to reverse the tendency (max+15 mM). Soluble proteins were not measured. Parallel to this observation, the water content in shoots of K-deprived plants was lower, which is also commonly observed under K deficiency and emphasizes the role of K in plant–water relationships (Mengel and Arneke 1982; Scherer et al. 1982; Leigh and Wyn Jones 1984; Leigh 1989; Jordan-Meille and Pellerin 2004).

Biomass accumulation was reduced for K-deprived plants from the fourth sampling date after K deprivation (119°C days) (Fig. 2). A significant decrease in leaf elongation rate was observed from 40 degree-days after commencement of starvation (Fig. 5). Conversely, a significant increase in the root elongation rate was observed between 45 and 75 degree-days after starvation (Fig. 7). Consistently a slight increase in the root/plant ratio was observed during an intermediate period after starvation (Fig. 3). The RUE did not differ between K treatments, except at the end of the experiment. The slight increase in the RER was associated with a slight increase in soluble sugar concentrations in axile root apices. A higher level of soluble sugars was also observed by other authors (Sharp et al. 1990; Caroll et al. 1994). These results suggest that the stimulation of axile root elongation during an intermediate period after K deprivation is the consequence of an increased allocation of carbohydrates. This interpretation is consistent with results of several authors who have shown that root elongation is controlled by carbohydrate availability (Muller et al. 1998). It is also consistent with the close correlation found between RER and the cumulative PAR during the three preceding days, which suggests that under our experimental conditions axile root elongation was limited by availability of carbohydrates. Since lateral root elongation is also very responsive to carbohydrate availability (Pagès 2000) their growth may have been stimulated during this intermediate period after K starvation. Unfortunately we did not collect data for laterals whose elongation occurred during the first 100 degree-days after starvation to confirm this assumption. However, after this intermediate period after K deprivation, characterised by reduced leaf elongation, a stimulated axile root elongation, a slightly enhanced root/plant biomass ratio and a constant RUE, almost all measured variables fell during the second part of the experiment. Table 4 summarizes the relative responses (i.e.

Table 4 Relative (K0/K+) growth (leaves, axile and lateral roots) and physiological (RUE, water content, sugar content) responses to K deficiency, towards thermal time, stage and K concentration in starved plants

Thermal time after starvation	Visible leaf stage	[K] in Plant DM_{K0}	[K] in plant water	Leaf Elongation Rate	Root Elongation Rate	RUE	Water content	Soluble sugars
°C days		g.g^{-1}	1 0.9 0.7 0.5 0.3 0.1	1 0.9 0.8 0.7	1.2 1.1 1 0.9 0.8 0.7 0.6 0.5 0.4	1 0.9 0.8 0.7	1 0.99 0.98 0.97 0.96 0.95	1.6 1.5 1.4 1.3 1.2 1.1 1 0.9 0.8 0.7
30	5,0	0.04						
60	5.9	0.034						
90	6.8	0.018						
120	7.8	0.015						
150	8.7	0.011						
180	9.6	0.009						



values of K0/values of K+) for most of these variables. On the whole, the plant response pattern during the initial period (moderate K deficiency) may be interpreted as the consequence of an initial effect of K deficiency on the ability of leaf growing zone to expand correctly. Conversely, photosynthetic processes don't seem to be affected. According to Lockart (1965), cell growth is controlled by turgor pressure and cell wall extensibility. Potassium is involved in the control of both processes, so that the observed reduced leaf elongation may be the consequence of altered cell-wall properties, osmotic and turgor pressures (Triboulot et al. 1997). These unfavourable biophysical conditions are not likely to affect the root compartment, especially in our hydroponic conditions (Sharp et al. 1988, 1990), whereas roots are likely to get more carbohydrates from shoots since their utilisation in aerial parts is limited by the reduced leaf growth. This latter interpretation is supported by four observations (1) root elongation was stimulated between 45 and 75 degree-days, whereas leaf elongation was reduced, (2) this stimulation was associated with an increased soluble sugar content. This higher sugar content may contribute to osmotic potential and provide carbon for growth, (3) in our experimental conditions (low incident PAR) root growth was likely to be limited by C, which is confirmed by the existence of correlations between RER and incident PAR, (4) the RUE was not affected by K treatments during the first part of the experiment, which suggests that net carbon assimilation per unit leaf area was not reduced, but its allocation between shoots and roots was altered, as confirmed by the root biomass/plant biomass ratio. This "unfavourable biophysical conditions" hypothesis and its associated effects on carbon allocation, which seems sufficient to account for the plant response during the first part of the experiment, however failed to explain what happened during the second part of the experiment. The root elongation was reduced, both on axile roots and to a greater extent on first-order lateral roots, and the RUE was severely reduced, which suggests that net carbon assimilation per unit leaf area was affected. Other physiological processes are likely to be affected, possibly due to the biochemical functions of K.

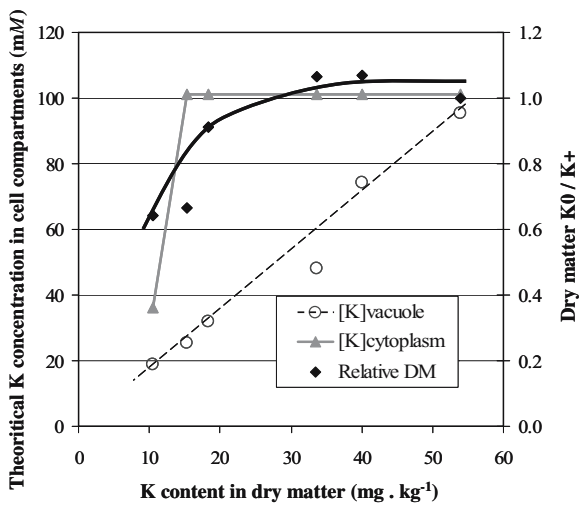


Fig. 8 Relative dry matter yield (DM K0/DM K+) and calculated concentrations of K in the cytoplasm and vacuole as a function of K content in the dry matter (mg kg^{-1}). Figure adapted from Leigh and Wyn Jones 1984, with the assumptions that $[K]_{cyt}$=101 mM, $[K]_{min\ vac}$=19 mM and that the proportion of intracellular volume occupied by the cytoplasm is 10%

This general pattern of response is in agreement with the model of Leigh and Wyn Jones, suggesting that biophysical functions, and therefore growth rates of organs, are likely to be affected first in the event of K deficiency. Moreover, it is worth noting that in our experiment the value of maize dry weight in relation to its K concentration followed the same trend as that reported by Leigh and Wyn Jones (1984) (Fig. 8). The expected concentrations in the vacuolar and cytoplasmic compartments were therefore calculated on the basis of Leigh and Wyn Jones' theoretical relationship, with the following assumptions: the proportion of intracellular volume occupied by the cytoplasm is 10%, the minimum level of K in the vacuoles is 19 mM and the initial value of K in the cytoplasm is 101 mM. This calculation suggests that the K content in the cytoplasmic compartment began to drop for K concentrations in dry matter below 15 g kg^{-1} (35 mM). According to the Leigh and Wyn Jones model, effects of K deprivation on K biochemical functions were thus expected below this threshold value. Consistently the "unfavourable biophysical conditions" hypothesis and its associated effects on carbon allocation failed to explain the plant response during the second half of the experiment (three last sampling dates), when the RUE was severely reduced for K0 plants. This roughly corresponds to the period for which the K concentration dropped below 35 mM (Fig. 1), as expressed on a tissue water basis.

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