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Original article

Contribution of different solutes to the cell osmotic pressure in tap and lateral roots of maritime pine seedlings: effects of a potassium deficiency and of an all-macronutrient deficiency

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Abstract – Seedlings of maritime pine (*Pinus pinaster* Ait.) were grown in hydroponics and submitted either to a potassium deficiency or to an all-macronutrient deficiency. In response to both nutrient stresses, tap root elongation was maintained while lateral root elongation was severely reduced. In both treatments, K content was decreased to 0.85 % of dry weight in roots and in shoots. Other minerals were little affected by the single deficiency except nitrogen, whose content increased significantly in roots. Measurements of the concentrations of inorganic ions, soluble sugars and amino acids on a tissue water basis revealed that, in unstressed plants, potassium, phosphate, choride, glucose, fructose and glutamine accounted for about two thirds of cell osmotic pressure with relative contributions depending on location in the root system. In seedlings subjected to deficiency, K was more or less efficiently replaced by soluble sugars, glutamine and/or sodium according to location in the root system. Osmotic pressure was better maintained in younger tissues but also in tap root tip as compared to lateral root tip.

potassium deficiency / osmotic pressure / inorganic ion / glutamine / soluble sugar / root growth

Résumé – Contribution de différents solutés à la pression osmotique cellulaire dans le pivot et les racines latérales de semis de pin maritime. Effets d'une carence en potassium et d'une carence en tous macroéléments. Des plantules de pin maritime (*Pinus pinaster* Ait.) cultivées en hydroponie ont été soumises à une carence en potassium et à une carence en tous macroéléments. En réponse aux deux stress nutritifs, l'élongation du pivot a été maintenue alors que celle des racines latérales a été fortement réduite. Le contenu en K a été réduit à 0,85 % du poids sec dans les racines et les parties aériennes. Les autres minéraux ont été peu affectés par la monocarence excepté l'azote, dont la teneur a augmenté significativement dans les racines. La mesure

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des concentrations en ions inorganiques, sucres solubles et acides aminés (par rapport à la teneur en eau) a montré que, chez les témoins, les solutés potassium, phosphate, chlorure, glucose, fructose et glutamine représentaient environ deux tiers de la pression osmotique cellulaire. Cependant, la contribution de ces éléments variait d'un endroit à l'autre du système racinaire. Dans les plantules carencées, le potassium a été plus ou moins efficacement remplacé par les sucres solubles, la glutamine et/ou le sodium en fonction de la position dans le système racinaire. La pression osmotique a été mieux maintenue dans les tissus jeunes mais aussi dans l'apex du pivot par rapport à l'apex des racines latérales.

carence en potassium / pression osmotique / ion inorganique / glutamine / sucre soluble / croissance racinaire

Abbreviations: Solute charges were not expressed in the text or in figures and tables: K, Na, Mg, Ca, Cl, PO_4 and SO_4 were used instead of K^+, Na^+, Mg^{2+}, Ca^{2+}, Cl^-, (PO_4^{3-}, HPO_4^{2-}, $H2PO_4^{-}$) and SO_4^{2-}. Moreover, [K] was written instead of 'potassium concentration' and similarly for other solutes.

K, potassium; MD, all-macronutrient deficiency; KD, potassium deficiency; TR, tap root; LR, lateral roots; TRA, tap root apex; TRPA, tap root post-apex; LRA, lateral root apices; LRPA, lateral root post-apices; P, turgor pressure; π, osmotic pressure; PAR, photosynthetically active radiation.

1. INTRODUCTION

Potassium (K) is the most abundant cation in plant tissues and plays both biochemical and biophysical roles in cells. In the cytoplasm, although it is not part of the structure of any plant molecule, it is required for the activation of several enzymes, for protein synthesis and photosynthesis. It also plays an important role in the vacuole where it contributes largely to the osmotic pressure and thus to the turgor pressure [11, 13; and references therein]. K deficiency may occur in trees growing on peaty or sandy soils [5, 20]. It has also been shown that, in nurseries, K deficiency, aggravated by an excess of nitrogen fertilization, caused injuries to *Picea pungens glauca* [2].

The most important consequences of K shortage are a higher sensitivity to frost damage, lower osmotic adjustment capacity during drought and reduced growth rate [13]. In contrast to nitrogen or phosphorus deficiencies, K deficiency induces a decrease of the root/shoot biomass ratio, which is due to a stronger reduction of root expansion [6]. A recent study conducted on maritime pine seedlings showed that a potassium deficiency (KD) affected differently the elongation of the different types of roots [23]. The elongation rate of the tap root (TR) was not affected while that of lateral roots (LR) was severely reduced. Furthermore, the effects on osmotic and turgor pressures (π and P) varied with location in the root system. In particular, π was significantly reduced in the mature cells next to the expanding zone of LR but not of TR. This suggested heterogeneous capacities of the root system to maintain π. These differences highlight the variability of behaviour existing within a root system, even at an early stage of development. Several studies have already shown that responses varied with the stimulus and with the type of roots. For instance, growth of LR of cotton seedlings was more inhibited by salinity than was primary root growth [19]. TR growth of *Phaseolus* remained almost constant during the night (as compared to the day) while LR growth was reduced [27]. On the other hand, temperature inhibited TR growth of soybean seedlings but did not affect LR growth [22]. In a recent study the osmotically active solutes in the maize root tip were mapped [17]. However, little information is available about

their distribution in various parts of the root system.

The aims of the present investigation were to answer several questions raised by the different growth and water relation responses of pine roots to a K deficiency [23]. i) What are the osmotically active compounds in pine root tissues? ii) Are their respective contributions to the osmotic pressure similar everywhere in the root system? iii) What are the effects of a potassium deficiency on the distribution of the solutes in the different parts of the root system? iv) Which solute(s) replace potassium?

Seedlings of *Pinus pinaster* Ait. were grown in conditions similar to those in our previous work [23] and concentrations of inorganic ions, soluble sugars and amino acids were determined on a water basis in different parts of the root system and related to cell osmotic pressure. Moreover, the potassium deficiency was compared with an all-macronutrient deficiency. In order to compare their effects with other data, the mineral contents of the seedlings in above- and below-ground parts were also determined on a dry matter basis.

2. MATERIAL AND METHODS

2.1. Plant material and growth conditions

Pinus pinaster Ait. seeds (provenance 'Landes', southwestern France) were grown in hydroponics as described previously [23]. The composition of the control nutrient solution was: $CaCl_2$ 0.5 mM, $MgSO_4$ 0.5 mM, KH_2PO_4 1 mM, NH_4NO_3 4 mM and micronutrients [21]. In the growth chamber, temperature was 22/19 °C, humidity 70/90 % (day/night), photoperiod was 16 h and the PAR was 500–600 μmol m^{-2} s^{-1}. The nutrient solution was changed once a week and pH was adjusted daily to 4.5–5.0 with NH_4OH.

Seedlings were subjected to two different mineral constraints. The first was a reduction of the K supply to 1/40th of the control level. In the nutrient solution, 1 mM KH_2PO_4 was replaced with [1 mM $(NH_4)H_2PO_4$ + 25 μM KH_2PO_4] and NH_4NO_3 supply was reduced from 4 to 3 mM in order to keep the NH_4^+ concentration at the level of controls. This treatment is referred to as KD. The second constraint consisted of a deficiency of all macronutrients (referred to as MD). Supplies of Ca, Cl, Mg, S, K, P and N were reduced to 1/40th of the control levels.

2.2. Harvest

Seedlings were harvested 30 days after germination. Lengths of the shoots (consisting only of a bunch of primary leaves), of the tap root (TR) and of the three longest lateral roots (LR; as an assessment of the length of the lateral roots) of each plant were measured just before harvest. After these measurements, plant root systems were rinsed by a rapid immersion in deionized water and quickly blotted dry.

To determine the mineral content as a fraction of dry weight, the whole root system and primary leaves were separated and dried at 60 °C for 48 h. To determine solute concentrations on a water basis, several parts of the root systems were collected: a) the apical 15 mm of the TR tip, referred to as TR apex (TRA); b) the following 30 mm of the TR, referred to as TR post-apex (TRPA); c) the apical 10 mm of the LR, referred to as LR apex (LRA); and d) the remaining part of the LR, referred to as LR post-apex (LRPA). For the KD stressed plants, no part d) could be collected since LR were usually shorter than 10 mm. Anatomical observations showed that parts a) and c) contained the expanding tissues but also some mature tissues [23].

The tissue samples were placed either in insulin-type syringes (for the inorganic ion analysis) or in 1.5 mL microtubes (for the soluble sugar and amino acid analysis), immediately frozen in liquid nitrogen and stored at –20 °C until analysis. Corresponding tissue samples of three to four plants were pooled in a single syringe (or microtube) in order to obtain enough material to carry out the analysis. Analyses were conducted on samples of 35–150 mg (15 mm of TR corresponded to about 12 mg fresh weight). All lateral roots of one plant were pooled together or split into two samples.

In order to increase the number of samples, the whole experiment was replicated twice. No differences appeared between the two replicates and therefore data were pooled together. In total, 56, 58 and 42 seedlings were used for the control, KD and MD treatments, respectively.

2.3. Mineral content as fraction of dry weight

Dry samples were ground to powder in liquid nitrogen. An aliquot of each sample (5 mg) was used to measure the total nitrogen content with a C.H.N. (Carlo Erba Instruments). Following the combustion of the sample at 950 °C, nitrogen oxides were reduced to N_2 and this gas was detected by a thermal conductivity detector. To determine K, Na, Mg, Ca and P contents, 20 mg of each sample were dry-ashed at 500 °C and ashes dissolved in 5 mL HCl 1N. Concentrations of S, P, Mg, Ca, Na and K were determined with a sequential ICP-OES (JY 38+, Jobin Yvon, Longjumeau, France) and expressed relative to dry weight (g g^{-1} DW). Because of the small volume of samples, we used the 'direct-picking' method with three replicates for each element.

2.4. Solute analysis in tissue extracts

2.4.1. Inorganic ion analysis

Insulin-type syringes (with a very small dead volume) were used to extract tissue sap. Glasswool, previously cleaned with HCl 1N, rinsed with ultra-pure water and dried, was placed at the bottom of each syringe. Severed tissue was inserted into the syringe tube and the piston put back into it. After thawing, tissue sap was collected by pushing the piston back and diluted with ultra-pure water about 100 times (determined by weighing). This brought ion concentrations into the range of the best accuracy of the methods of analysis.

Concentrations of K, Na, Mg, Ca and P were then measured with ICP-OES as described above, and of Cl^-, NO_3^-, PO_4^{3-} and SO_4^{2-} with ionic chromatography with a conductimetric detection and an autosuppression recycle mode (Dionex DX 300, Sunnyvale, USA). This was associated with an automatic injector (Gilson 222 XL, Villiers le Bel, France). Guard column AG12A and column AS12A were used with an (Na_2CO_3 2.7 mM / $NaHCO_3$ 0.3 mM) eluant and a flow rate of 1.5 mL min^{-1}. Injection volume was 50 µL.

We noticed that P concentrations measured by inductively coupled plasma were correlated with the PO_4^{3-} concentrations measured by ionic chromatography over the whole range of concentrations ($[PO4^{3-}] = 1.02[P] - 2.24$, $r^2 = 0.94$, data not shown). A similar correlation was observed between S and SO_4^{2-}, indicating that soluble P and S in the tissue extracts were present in inorganic form.

2.4.2. Soluble sugar analyses

Frozen tissues were crushed in a tube containing 0.5 mL of 80 % ethanol at 80 °C. These conditions neutralized invertase before it could decompose sucrose into fructose and glucose. Microtubes were rinsed with 0.5 mL 80 % ethanol. After 30 min extraction, supernatants were collected and residues rinsed twice with 0.5 mL 80 % ethanol. After drying, the extracts were dissolved in 1 mL ultrapure water, purified with micro-columns filled with ion-exchange resins (0.5 mL cationic resin, Amberlite, IRN77, Prolabo; 0.5 mL anionic resin, Ag1×8, formate, Biorad) and dried again. Before analysis, the extracts were dissolved in 400 µL ultra-pure water and filtered (0.45 µ, Acrodisc, Gelman). Next 20–40 µL were injected in a HPLC equipped with a Polysphere Pb column (Merck) and ultrapure water as eluant.

2.4.3. Amino-acid analysis

Extraction was carried out at 4 °C. 40 µL of an internal standard (α-butyric acid) were added to samples which were crushed with a pinch of pure quartz sand in 150–300 µL of 70 % methanol. After a 15-min incubation, microtubes were centrifuged for 10 min at 14 000 r/min. Supernatants were collected and the residues rinsed with 150 µL 70 % methanol. The extracts were filtered (0.45 µm) and, 90 s before the injection in HPLC, 10 µL ortophtaldialdehyd (OPA) were added to 40 µL sample. The fluorescent derivatives of the amino acids were detected at 340 nm. The HPLC was fitted with a RP18 column and a (20 % methanol–80 % sodium acetate)–100 % methanol gradient was used as eluant.

2.5. Calculation of the cellular concentration of the solutes

In a side experiment on unstressed plants, we measured the osmotic pressure of single cells of the different parts of the root (TRA, TRPA and LRA) with a cryoscopic picolitre osmometer [12] and the osmotic pressure of the sap of these tissue parts with a vapour pressure osmometer (Wescor 5500). The ratio between cell osmotic pressure and tissue sap osmotic pressure yielded a coefficient corresponding to the dilution of cell sap by apoplasmic sap or by water remaining on the surface of the roots. The dilution coefficients were 1.15, 1.28 and 1.75 for TRA, TRPA and LRA, respectively. The large dilution coefficient for LRA was probably due to the drying technique used for these sections (several roots dry-blotted together, in order to limit root dehydration before storage). The coefficient determined for LRA was also used for LRPA sections.

Cellular solute concentrations were calculated by multiplying the concentrations measured in tissue sap by the dilution coefficient of the corresponding root section. In order to calculate the contribution of each solute to the cell osmotic pressures (π), these concentrations were related to π measured in the corresponding tissue sections in plants grown in same conditions as described above [23]. Cell π was measured by cryoscopy and converted from MPa to osmol L^{-1} using the Van t'Hoff relation [9]. When calculating the contributions of solutes to cell π, we neglected the osmotic coefficients and thus obtained semi-quantitative contributions of solutes to π.

3. RESULTS

3.1. Effect of deficiencies on growth and mineral content

The potassium deficiency (KD) did not affect tap root (TR) elongation but reduced significantly lateral roots (LR) elongation of the maritime pine seedlings (*figure 1B* and *C*), as found in our previous experiment [23]. Moreover, growth of shoots, displaying only a bunch of primary leaves, was significantly decreased (*figure 1A*) and symptoms of K deficiency, such as yellowing and necrotic rings, appeared on needles. As compared to KD, the all-macronutrient deficiency (MD) inhibited LR elongation less and shoot growth more but did not induce any deficiency symptoms.

In control plants, K content was larger in roots than in primary leaves, 2.4 and 1.7 %DW, respectively (*figure 2*). K content decreased uniformly to 0.85 %DW in response to both mineral constraints. Na content increased significantly but remained below 0.3 %DW since this element was only supplied with the micronutrient solution (0.1 mM FeNaEDTA). Roots accumulated more Na than shoots. Ca, Mg and P contents were little affected by KD and N content remained unchanged at about 4.5 %DW in primary leaves but was significantly increased from 3.9 to 4.8 %DW in roots. MD decreased significantly Ca, Mg, P and N contents both in roots and primary leaves.

3.2. Solute contribution to cell osmotic pressure in unstressed plants

In all parts of the roots, K was the main cation (62–107 mM) and Cl and PO_4 the main inorganic anions with a Cl/PO_4 ratio of about two (*figure 3*). [Na] remained below 5 mM. [Ca], [Mg], [NO_3] and [SO_4] were less than 2 mM, contributing very weakly to cell osmotic pressure (π), and thus were not presented in *figure 3*. K, Na, Cl and PO_4, contributed approximatively half of cell π (*table I*). Osmotically active organic compounds were glucose and fructose, with a 1:1 ratio, and glutamine, present in much larger concentration than the other amino acids. Sucrose was present in these tissues as traces only although significant concentrations were found in older roots (data not shown).

Solute concentrations differed slightly between the parts of the roots. Most important points were: higher [soluble sugars] in

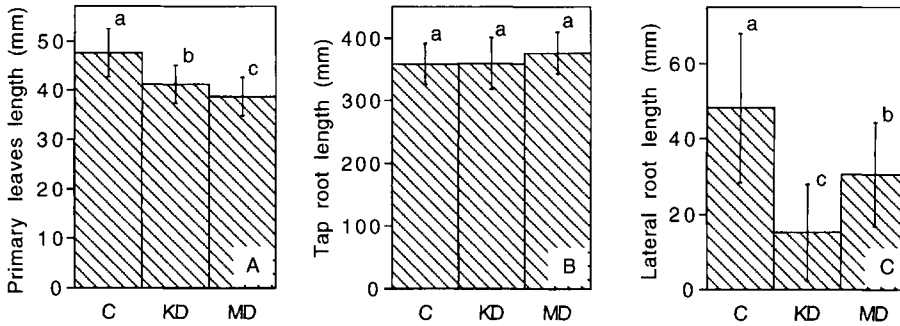

Figure 1. Effects of a potassium (KD) and an all-macronutrient (MD) deficiency on the length of A) primary leaves, B) tap root and C) lateral roots (mean of the three longest) of 1-month-old maritime pine seedlings. Means ± SD, ($41 \leq n \leq 58$). Different letters indicate significantly different means (ANOVA followed, if significant, by Bonferroni multiple-comparison tests, 5 %).

apices than in more mature tissues (*figure 3* and *table I*); higher [glutamine] in TR than in LR; lower [organic solutes] and higher [inorganic ions] in LR than in TR. Globally, solutes analysed in this study contributed to 65–84 % of cell π (*table I*).

3.3. Effect of deficiencies on the contribution of solutes to the osmotic pressure

In TRA, none of the deficiencies changed the osmotic pressure and the fraction of π due to the identified solutes remained also constant (*figure 4A* and *table I*). However, KD reduced [K] from 83 to 28 mM and, more surprisingly, this was associated with a decrease of [Cl] although its supply was not modified. An increase of [glucose], [fructose] and [glutamine] fully compensated for the deficit of inorganic solutes. MD reduced [K] less severely than did KD (to 50 mM) although limitation of K supply was similar in both treatments (*figure 2*). The concommitant [Cl], [PO_4] and [glutamine] decreases were compensated for by an increase of [soluble

Table I. Effects of a potassium (KD) and an all-macronutrients (MD) deficiency on the contribution of different solutes to the cell osmotic pressure (%) in different parts of the root system.

	Tap root						Lateral roots		
	Apex (0–15 mm)			Post-apex (15–45 mm)			Apex (0–10 mm)		
	C	KD	MD	C	KD	MD	C	KD	MD
Soluble sugars	26	32	40	14	27	28	13	12	30
Glutamine	7	16	5	11	28	7	5	7	5
K	23	8	14	19	3	9	33	4	10
Inorganic ions (including K)	42	22	28	40	21	31	66	35	43
Total	75	70	73	65	76	65	84	54	78

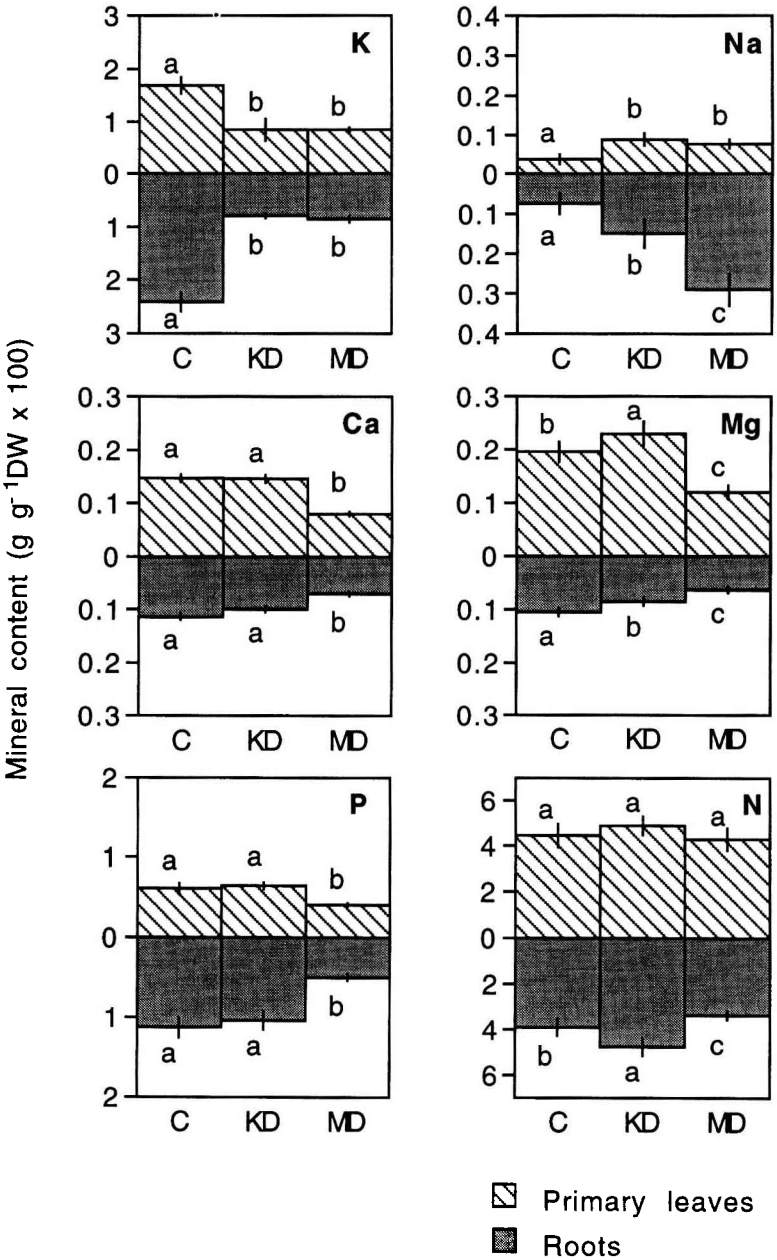

Figure 2. Effects of a potassium (KD) and an all-macronutrient (MD) deficiency on potassium, sodium, calcium, magnesium, phosphorus and total nitrogen content (%DW) in primary leaves (upper part of the graphs) and roots (lower part of the graphs) of 1-month old maritime pine seedlings. Means ± SD, ($6 \leq n \leq 8$). Different letters indicate significantly different means (ANOVA followed, if significant, by Bonferroni multiple-comparison tests, 5 %).

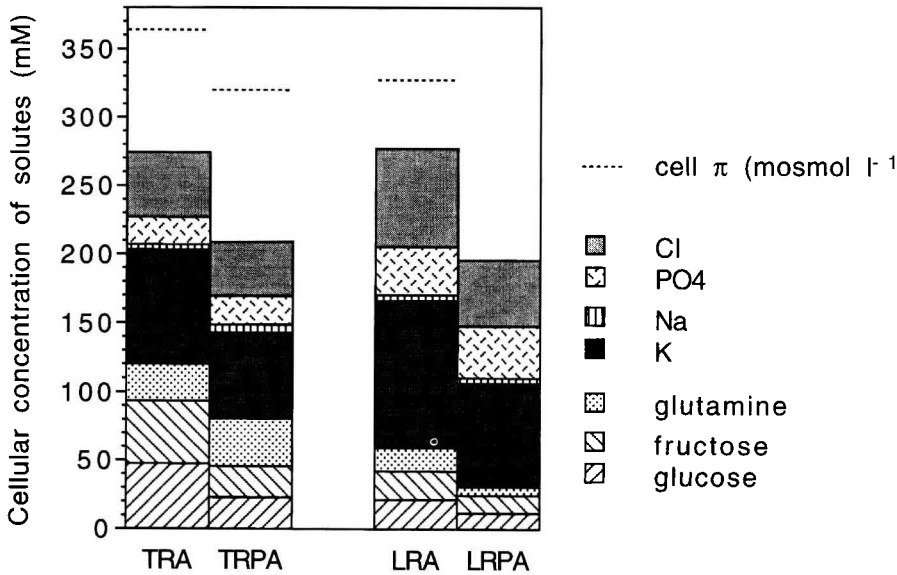

Figure 3. Cellular concentrations of glucose, fructose, glutamine, potassium, sodium, phosphate and chloride and cell osmotic pressure in different parts of the root system of 1-month old maritime pine seedlings (TRA, tap root apex; TRPA, tap root post-apex; LRA, lateral root apex; LRPA, lateral root post-apex).

compensated for by an increase of [soluble sugars].

In LRA, the KD treatment dramatically reduced [K] from 107 to 10 mM and also affected significantly [Cl] (*figure 4B*). By contrast to what happened in TRA, [soluble sugars] and [glutamine] were not significantly modified and [Na] increased from 4 to 16 mM. Although cell π was reduced, the 'explained' fraction of π decreased (*table I*). This means that solutes other than those analysed here contributed to π maintenance. In response to MD, [K] was reduced to 25 mM, which is less than by KD as also happened in TRA (*figure 4A*). [Cl] and [PO_4] were reduced as compared with control plants and [soluble sugars] and [Na] increased largely. Cell π decreased and the fraction of 'explained' π remained almost constant (*table I*). Surprisingly, the ratio [glucose]/[fructose], which was 1 for all samples in the control and KD treatments, was 1.6 in MD.

In TRPA, KD reduced [K] from 62 to 9 mM, that is to a level similar to that in LRA (*figure 4C*). There were increases in [glucose], [fructose] and, more importantly, [glutamine] which compensated for more than the decreases in [K] and [Cl]. In MD plants, the deficit of K was compensated for by Na and soluble sugars, as in LRA. In response to both treatments, π was slightly decreased and the fraction of π due to the solutes analysed remained unchanged or was slightly increased (*table I*).

4. DISCUSSION

In *Pinus pinaster* seedlings, potassium concentrations ([K]) found in root cells

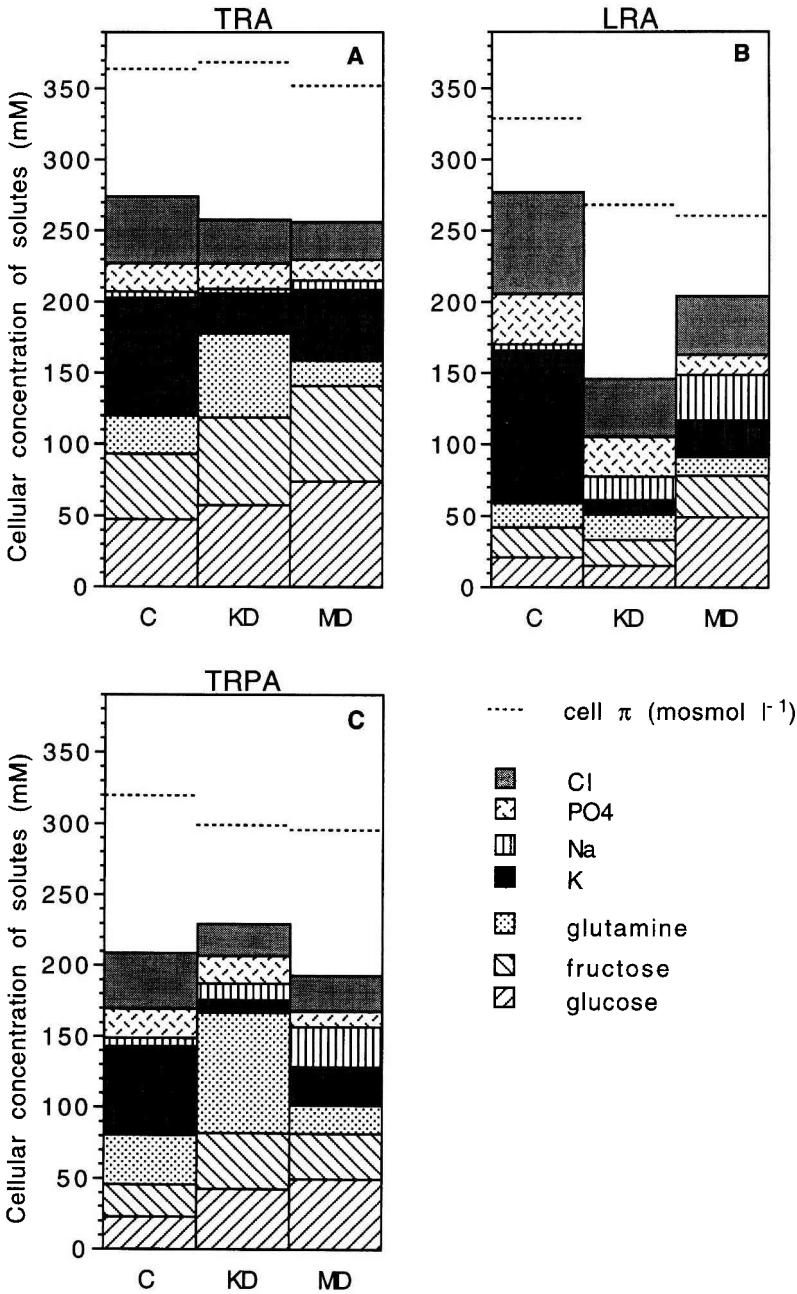

Figure 4. Effects of a potassium (KD) and an all-macronutrient (MD) deficiency on the contribution of glucose, fructose, glutamine, potassium, sodium, phosphate and chloride to the cell osmotic pressure in A) the tap root apex, B) the lateral root apex and C) the tap root post-apex of 1-month old maritime pine seedlings.

(62–107 mM) were close to those measured in the same species (80 mM, [15]), in maize roots (60–97 mM, [16]; 75 mM, [17]) and slightly lower than in barley roots (about 160 mM, [28]). The calculation of cell [K] from tissue [K] gave results similar to those measured directly in cells by energy dispersive X-ray microanalysis [16, 17] or by microelectrodes [28]. The good correlation between the range of [K] found in the present study and those found in the other studies suggests that the cation exchange capacity of the cell wall was probably low.

Major inorganic solutes, K, PO_4 and Cl, accounted for approximately half the cell osmotic pressure (π). In comparison, they accounted for 57 % in maize roots [16] and for only 20 % in tissue extracts of white apices of oak roots [25]. It seems that the fraction of π due to inorganic solutes is higher in leaves than in roots: 45 % in oak [25] and above 90 % in barley [8]. As in maize roots [16], glucose and fructose were found at appreciable concentrations and sucrose only as traces. In a control experiment, known quantities of sucrose were added to samples and were recovered, showing that it was not hydrolysed during the extraction and purification steps and thus that the 1:1 glucose/fructose ratio was not an artefact. The higher soluble sugar concentration in the apices is probably related to the intense cell division and expansion in the growing zone. The other important solute, glutamine, reached high concentrations (35 mM). This solute plays a role in nitrogen storage and transport and here contributed to cell π, especially in the mature zone of the tap root. Inorganic ions, soluble sugars and glutamine accounted for about two third of cell π. The remaining part of π could be due to ammonium, other amino acids [17, 26] or organic acids. Indeed, quinate, succinate and malate are present in leaves and roots of oak [24]. This hypothesis is supported by the presence of a large peak with the same retention time as shikimate on the anions chromatograms. However, identification tests were not made and no conclusion could be drawn.

In the control and MD treatments, the charge balance was close to unity or presented a slight deficit in negative charges (data not shown). Organic anions may carry the missing negative charges. In contrast, in the KD treatment, there was a strong deficit in positive charges, showing that one or several cations were not taken into account. One of them may be ammonium which cannot accumulate in the cytosol but could be present in the vacuole at high concentration (50 mM) as found by Lee and Ratcliffe [10] in maize root tissue.

Potassium deficiency (KD) reduced the K content of roots and shoots by a factor of about 3 to 0.85 %DW and induced visible symptoms of deficiency. In birch seedlings, the minimal K content still allowing maximal growth was about 1.2 %DW [7]. In Scots pine needles, it was lower, close to 0.5 %DW, but was measured in an adult stand [5, 20]. By contrast to what happened in the observations on birch, KD significantly increased total N content in maritime pine roots. Although the mineral contents in MD were similar to or lower than those in KD plants, no visual deficiency symptoms could be seen on MD plants. This may be due to a better balance between minerals.

Although most nutritionists express mineral contents on a dry matter basis, Barraclough and Leigh [4] underlined the importance of expressing them on a tissue water basis, especially for K because of its importance in plant–water relations. Furthermore, [K] (in mM) changes less during plant development than K in %DW and has been shown to be independent of the N and P supplies. However, caution should be taken since tissue water content varies with water availability and also

depends on K content [4, 7]. In the present study, expressing solute concentrations on a water basis allowed us to analyse their contribution to π. Osmotic coefficients were ignored when calculating the contribution of solutes to π since, considering the inherent inaccuracies in the measurements of concentrations, the corrections would not have been significant. Moreover, osmotic coefficients are more or less unknown for such complicated solutions.

In response to KD and MD, K was replaced by soluble sugars and/or glutamine and/or Na. No other cations, e.g. Ca or Mg, played a role as alternative osmoticum. This is in contrast to what happened in leaves of *Phaseolus* [14] but in agreement with results of Barraclough and Leigh [4] where Na was more efficient than Ca and Mg in replacing K and maintaining yield of ryegrass. In response to KD, mainly organic substitutes were involved. According to Leigh and Wyn Jones [11; and references therein], the glutamine accumulation may have been due to an inhibition of protein synthesis. When all macronutrients were reduced, π maintenance involved accumulation of soluble sugars and Na.

The maintenance of π occurred more or less efficiently and involved different solutes, depending on the part of the root considered. By contrast to the post-apex of the tap root (TRPA) and to the apex of the lateral roots (LRA), the apex of the tap root (TRA) seemed to be protected: [K] decreased less, π was perfectly maintained and there was no Na accumulation. Logically, younger and more active tissues appeared to be favoured over more mature ones. This has also been noticed in poplar where, following K deficiency, [K] was better maintained in root apices [18]. More surprisingly, but in agreement with the growth and water relation parameter responses [23], solute concentrations in the root apices (TRA and LRA) were very differently affected. The biophysical analysis conducted in the previous study suggested that a reduction of wall extensibility was involved in the inhibition of LR growth. However, turgor pressure (P) and π in cells next to the expansion zone were more sensitive to the mineral constraints in LR than in TR. This could have had effects additive to the reduction of wall extensibility. Indeed, Mengel and Arneke [14] related the reduction of leaf expansion of *Phaseolus* in response to a K deficiency to a reduction of cell P and π. Similarly, inhibition of ryegrass growth induced by K deficiency was associated with a reduction of π [4].

In pine seedlings, the different composition of cell sap in TR and LR and the modifications induced by the mineral constraints may be related to different functions of these two types of roots. Many studies have reported heterogeneous behaviour or different functions of individual roots within a root system, even in the absence of evident morphological differences [27; and references therein]. In several cases, for instance considering the responses to temperature [22] or to light [27], it was suggested that differences in carbon allocation to TR and to LR could be involved. Moreover, it has been established that root elongation is tightly dependent on carbon assimilation [1] and that potassium deficiency inhibits photosynthesis and reduces carbon availability in roots [6]. According to Atzmon et al. [3], in case of a shortage of assimilates in the root system, the apical dominance of TR becomes more significant at the expense of LR. The growth response of TR and LR to KD seems to confirm that TR presents a stronger carbon sink. Moreover, solute distribution between LR and TR following KD indicates that sink strength for K was similar to sink strength for carbon. The question whether these are independently regulated remains unanswered.

Solutes contributing to cell osmotic pressure were heterogeneously distributed in the root system of maritime pine seedlings. Moreover, potassium and all-macronutrient deficiencies induced different responses in tap and lateral roots in terms of growth, solutes involved in K replacement and efficiency in maintenance of turgor and osmotic pressures. This illustrates that the morphological differences between TR and LR are associated to physiological differences. The complexity of the root system is still poorly understood and further studies on the variability of the responses to various constraints within the root system are needed.

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