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Solubilization of rock phosphate by rape

II. Local root exudation of organic acids as a response to P-starvation

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Abstract

Local rhizosphere acidification by rape as a reaction to P-starvation was visualized by means of an agar plate technique. By means of a modification of this technique local differences in cation-anion uptake and organic acid exudation along intact roots of rape were observed for plants grown on nutrient solution with or without added P. No differences in uptake rates of K-, NO_3- and Ca-ions could be detected between P-starved and P-supplied plants. However, exudation of malic and citric acid was distinctly higher in acidified root zones of P-starved plants, coinciding with higher levels of malate in the corresponding root tissue. Organic acid exudation is indicated as the cause of local rhizosphere acidification by rape as a reaction to P-starvation and as a possible mechanism of its phosphate-solubilizing capacity.

Introduction

Unlike most other nonmycorrhizal species, rape (*Brassica napus*) is able to grow with rock phosphate as P-source, even when NO_3 is the N-source. In a previous paper (Hoffland *et al.*, 1988) we provided evidence that this property cannot be explained by rhizosphere acidification due to an over-all imbalanced cation-anion uptake (Hedley *et al.*, 1982; 1983), nor by high Ca uptake (Bekele *et al.*, 1983). Therefore, other mechanisms, which are independent of nutrient uptake, have to be regarded.

Rhizosphere acidification in relation to mineral nutrition has been reviewed by Marschner *et al.* (1986). Local rhizosphere acidification has been shown for a number of dicotyledonous species as a reaction to Fe-deficiency and for lupin as a response to P-deficiency. Gardner *et al.* (1983) demonstrated that the proteoid roots of P-deficient lupin plants secreted large quantities of citric acid. P-starved alfalfa seedlings also exuded organic acids (Lipton *et al.*, 1987) but in the latter case it is unknown whether this is restricted to certain root zones. Moorby *et al.* (1988) demonstrated that P-starved rape acidifies its rhizosphere only just behind the root tip, but suggested that this was due to a localized shift in ion uptake. In earlier experiments, no significant quantities of organic acids have been detected in the rhizosphere of rape (Hedley *et al.*, 1982).

In this paper, we present the results of experiments dealing with the occurrence of local exudation of organic acids from P-starved rape plants, being a potential mechanism underlying their capacity to mobilize rock phosphate.

Materials and methods

Growth of plants

Seeds of rape (*Brassica napus* cv. Jetneuf) germinated in moist quartz sand were after 6 days transferred to a nutrient solution with or without 0.25 m*M* KH_2PO_4. The nutrient solution consisted

of: 1.25 m*M* $Ca(NO_3)_2$, 1.25 m*M* KNO_3, 0.50 m*M* $MgSO_4$ and trace elements (in $mg\,l^{-1}$): Fe (as FeEDTA) 4.6; B 0.5; Zn 0.05; Cu 0.02; Mo 0.01. After 7 days of growth in a growth chamber on nutrient solution, the plants were used for experiments. At that moment, clear symptoms of P-deficiency were visible. Growth conditions: day/night regime 16/8 h; light intensity $70\,W\,m^{-2}$; temperature 20 °C; relative humidity ± 80%.

Visualization of rhizosphere acidification

To visualize acidification and/or alkalinization along single roots of intact + P and − P rape plants in situ, an agar technique, similar to that described by Weisenseel *et al.* (1979), was applied. After having been laid out on a glass plate, the roots were covered with a 2–3 mm thick agar layer. Bromocresol purple (0.015%) was used as pH indicator, dissolved in an agar medium (1.0% agar) containing the normal nutrient solution without phosphate. The solution was adjusted to pH 5.8 with NaOH and kept liquid at 45 °C before being poured over the roots. Acidification and/or alkalinization became visible within 1 h.

Collection and analyses of root exudates

Root exudates were collected by means of an adapted agar plate technique. While spreading the roots on a glass plate, three lateral roots were placed next to each other, with the root tips in adjacent positions (Fig. 1). Before covering the roots with agar solution, small plastic rings (ϕ 1.2 cm) were placed over the root zone just behind the root tips, and another one over the same three roots, as closely as possible to the root base. After covering the roots outside the rings with agar solution, 0.25 ml − P nutrient solution was pipetted into the rings. After 2 h incubation at room temperature and high humidity, the contents of the rings were collected ("root tip" and "root base" separated) and immediately analyzed for citrate, malate and fumarate by enzymatic procedures (Anonymous, 1984).

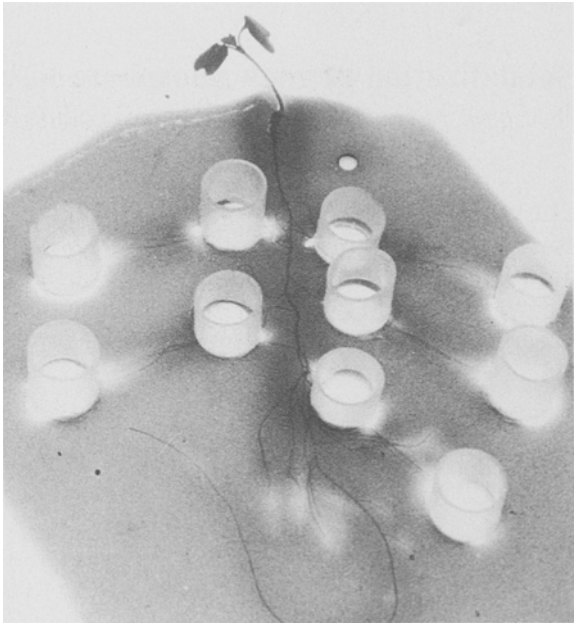

Fig. 1. Method used to determine exudation of organic acids and ion uptake at different distances from root tips of intact plants. The plants were partly covered with an agar solution.

Uptake of K-, Ca- and NO_3-ions by root tips in situ

About the same procedure as described for root exudates was followed. Larger rings (ϕ 1.8 cm) were used, containing 0.5 ml − P nutrient solution (see above). After 24 h incubation, the contents of the rings were analyzed. Quantities absorbed by the zone just behind the root tip of − P and + P plants were compared. The uptake rate was assumed to be constant in time. K and Ca were determined by flame photometry and NO_3 by automatic spectrophotometry after reduction to NO_2.

Internal malate and citrate concentrations in root sections

From plants grown on − P and + P nutrient solution, two root sections were collected: one 0.0–1.5 cm and another 1.5–3.0 cm behind the root tip. After collection of about 25 mg dry weight, the samples were ground, extracted in 15 ml demineralized water and these extracts were analyzed for malate and citrate.

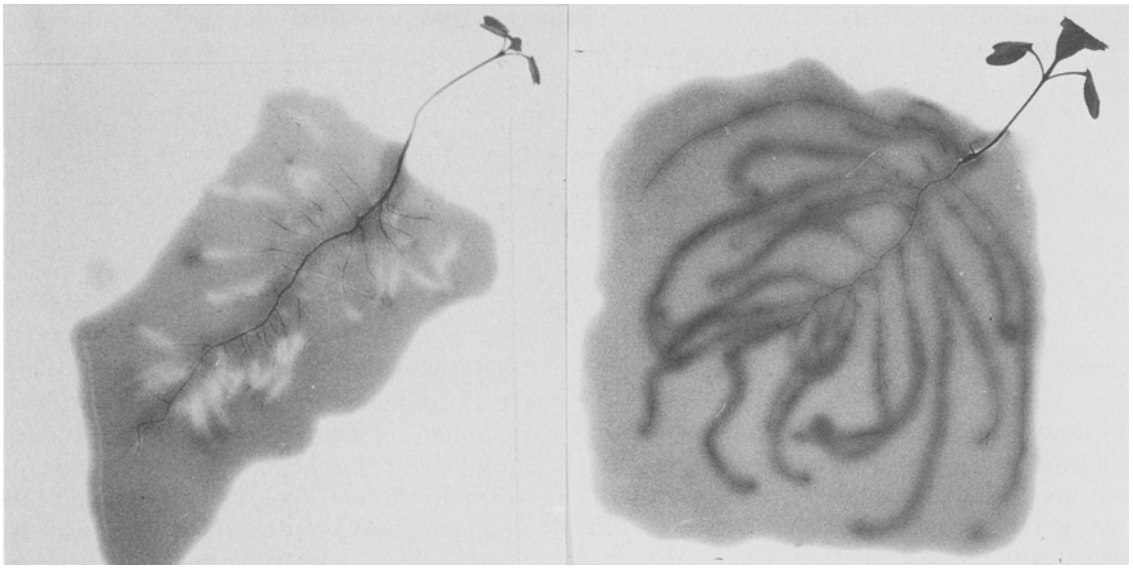

Fig. 2. Alkalinization (dark) and/or acidification (light) of a thin agar layer covering roots of rape plants grown for 7 days on nutrient solution without P (*left*) or with P (*right*). The agar (pH 5.8) contained bromocresol purple and a −P nutrient solution.

Results

Changes in pH on agar plates depending on the plant P-status

Within a few hours, clear yellow zones could be detected in the agar plates with plants grown without P (Fig. 2). The acidification was limited to a root zone of about 1.5 cm length, just behind the root tip. After 22 h incubation the yellow spots could have increased to spots of about 2 cm diameter. Along the remainder of the −P root system, only alkalinization could be detected. No acidification occurred along the +P roots (Fig. 2).

Table 1. Amounts of organic acids detected after 2 h in exudates from different root zones of rape plants, grown for 7 days on nutrient solution with or without P. Values are means ± sd (n = 9)

P-status	Root zone	Amounts of organic acids in exudates after 2 h, nmol cm^{-1} root	
		Malate	Citrate
−P	Behind root tip	0.87 ± 0.11	0.27 ± 0.15
	Near root base	0.20 ± 0.10	0.13 ± 0.10
+P	Behind root tip	0.15 ± 0.09	0.06 ± 0.03
	Near root base	0.03 ± 0.02	0.01 ± 0.02

Exudation of organic acids

Malate and citrate were detected in the exudates of −P and +P plants (Table 1). The amounts in exudates of −P roots just behind the root tips were significantly higher than those of the other zones. No fumarate could be detected.

Uptake of K-, Ca- and NO_3-ions by root tips in situ

For K, Ca and NO_3, uptake rates of +P plants were about twice as high as those for −P plants (Table 2). However, for the electrical charge balance these differences did not have any consequence (Table 2).

Internal organic acid concentrations of two root zones

The malate and citrate tissue concentrations of root zones 0.0–1.5 cm behind the root tip of −P plants were clearly higher than those of the zone further from the tip (Table 3). Generally, the malate and citrate concentrations of +P plants were lower than those of −P plants.

Table 2. Amounts of nutrients taken up during 2 h by a 1.8 cm root zone behind the root tip of rape plants, grown for 7 days on nutrient solution with or without P. Values are means ± sd (n = 14)

P-status	Uptake of ions			
	(nmol cm^{-1} root)			(neq cm^{-1} root)
	K	Ca	NO_3	$\Sigma(K + Ca - NO_3)$
−P	1.1 ± 0.3	1.9 ± 0.3	5.6 ± 0.7	−0.8 ± 0.4
+P	2.5 ± 0.6	3.4 ± 0.3	10.1 ± 1.4	−0.8 ± 0.6

Discussion

In case of NO_3 nutrition, the uptake pattern of rape grown with P is acidic, *i.e.* more anions than cations are taken up (Hoffland *et al.*, 1988). Alkalinization of the agar along the roots (Fig. 2) is consistent with this phenomenon. The uptake pattern is not affected by P-starvation. Nevertheless, it has become clear that the rhizosphere of −P plants undergoes acidification, but only in a restricted root zone (Fig. 2; Moorby *et al.*, 1988).

On the basis of the ionic composition of the plants (Hoffland *et al.*, 1988), NO_3, K and Ca were regarded as the relevant nutrients with respect to rhizosphere acidification. Even in the acidified root zones (Fig. 2), no H-ion extrusion, caused by an excess of cation over anion uptake can be expected (Table 2). This is in contrast to the suggestion of Moorby *et al.* (1988) that local acidification might be due to a locally high Ca uptake. We observed that acidification also occurred when no nutrients were added to the agar solution. Therefore, local acidification caused by P-stress cannot be explained in terms of changes in nutrient uptake pattern along the root surface, which is consistent with earlier results (Hoffland *et al.*, 1988).

Table 3. Malate and citrate concentrations in different root sections of rape plants, grown for 7 days on nutrient solution with or without P. Values of 2 replicates (A and B) are given

P-status	Distance from root tip (cm)	Concentrations in roots (μmol g^{-1} dry matter)			
		Malate		Citrate	
		A	B	A	B
−P	0.0–1.5	256	178	84	78
	1.5–3.0	92	116	54	55
+P	0.0–1.5	35	32	9	13
	1.5–3.0	42	21	44	10

Organic acid exudation (Table 1), acidification of the rhizosphere (Fig. 2) and organic acid concentrations in the relevant root sections (Table 3), are all highest for the root tips of P-starved plants. When the effect of NO_3, K and Ca uptake (Table 2) is superimposed to the effect of organic acid exudation (Table 1), only in the root tips of P-starved plants a net acidification can be expected. This is in line with the results of the agar plate technique (Fig. 2). Thus, the major part of the local rhizosphere acidification by P-stressed rape plants has to be attributed to exudation of malate and, to a lower extent, citrate.

Exudation of soluble amino acids and reducing sugars induced by P-stress was demonstrated by Ratnayake *et al.* (1978) for sudangrass. They postulated that permeability of root membranes is increased during P-deficiency, due to decreased phospholipid levels. If this would be the explanation for the increased excretion of organic acids by P-stressed rape plants, then lower rather than higher root tissue concentrations would be expected. Our results (Table 3) suggest an increased rate of organic acid synthesis in P-stressed rape plants.

An increased root tissue organic acid concentration has been demonstrated for Fe-stressed bean plants (Landsberg, 1984). The local rhizosphere acidification in this case was caused by an extrusion of protons, in exchange for cations (Van Egmond and Aktas, 1977). The protons originated from organic acids (De Vos *et al.*, 1986). In contrast, in our P-stressed rape plants no increased cation uptake could be detected in the acidifying root zones (Table 2). Instead, organic acids were detected in the root environment (Table 1).

A high local exudation rate of organic acids may be of ecological benefit, compared with lower exudation rates along the complete root system. In well-buffered soils, a pH decline can only be

achieved by high flux densities of organic acids. Further, a strong decrease of soil pH may inhibit growth of microorganisms, and consequently prevent microbial degradation of the exuded substances. This view, which leaves no space for a possible favorable role of microorganisms in rock phosphate mobilization, is in agreement with the results of Laheurte and Berthelin (1988) and Hedley *et al.* (1982). The latter did not find any relation between the number of hydroxyapatite-solubilizing bacterial colonies and P-mobilization by rape. We have indications that microbial degradation of exudates has to be taken into account: in our samples malate and citrate were degraded within a few hours with concomitant CO_2 production. Microbial degradation may also be the reason why Hedley *et al.* (1982) did not find significant amounts of organic acids. Further research is necessary to establish the role of microorganisms in rock phosphate mobilization by rape.

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