Measurement of pH Gradients in the Rhizosphere*

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Introduction

Root exerts a dissolving effect on soil minerals in the rhizosphere (Jaillard, 1989a; Malquori et al., 1975; Hinsinger et al). Several processes are involved in this action. The most commonly accepted is the displacement of solubility equilibria by ionic absorption, but local acidification of soil certainly also contribute to this phenomenon. pH values in the rhizosphere result from metabolic processes (ionic absorption, exsorption of protons, CO_2 or organics acids) and reaction of soil components against these processes. Therefore they are related to the behaviour of the complex system soil-root-microorganisms and must be measured in these conditions.

Some methods were proposed for measuring pH at the soil/root interface (see Jaillard 1989b for a recent revue). The Weisenseel's one (Weisenseel et al., 1979) is based on the use of vital pH indicator dyes. This method has been used and improved by several authors (Marschner et al., 1982; Pilet et al., 1983). Its non-destructive character constitutes its main advantage. However the only visual observation of colour change does not allow pH quantification and moreover limits spatial resolution.

This work concerns an improvement of this method based on spectrodensitometric measurement of the colour change in order to reach accurate measurement of pH with a sufficient spatial resolution.

Method

Quantification of pH firstly implies the knowledge of the relation between pH value and optical density (O.D.). Structural modifications of the indicator molecules due to the reaction of protonation-deprotonation vs acidity conditions can be followed by

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spectrometry in buffer solutions. The corresponding spectra of the three vitals indicators generally used (bromocresol green, bromocresol purple and phenol red) are presented on Fig. 1. It can be observed that these spectra present an isobestic point, which demonstrates that there is an equilibrium between acidic and basic forms of the indicator and that the relation between pH and O.D. must be univocal.
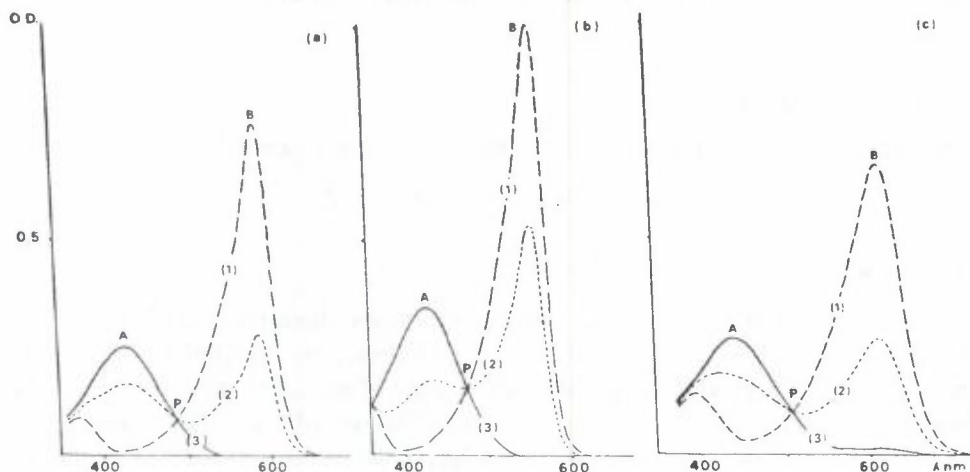

Figure 1. Absorption spectra of usual vital indicators for different values of pH. (P) isobestic point. Acidic (A) and basic (B) forms of the indicator. Indicator concentration 6mg/l, optical path: 1cm. (a) bromocresol purple: (1) pH 9, (2) pH 6, (3) pH 4. (b) phenol red: (1) pH 9.5, (2) pH 7.8, (3) pH 6.5. (c) bromocresol green: (1) pH 7, (2) pH 4.5, (3) pH 2.6.

Equilibrium between acidic (A) and basic (B) forms of the indicator can be written as :

$$K = (H^+) \cdot (B)/(A) \qquad \text{where ( ) means activity}$$

or
$$pH = pK + \log(B)/(A) \tag{1}$$

Given the wavelength and the indicator concentration, optical density D is related to optical densities D_A and D_B of the pure acidic and basic forms by :

$$D = (D_A \cdot [A]/[A] + [B]) + (D_B \cdot [B]/[A] + [B]) \tag{2}$$

Where [ ] means concentration.

For dilute solutions combining equations (1) and (2) gives then :

$$pH = pK + \log(D - DA)/(DB - D)$$

pK values determined on the basis of this relation with solutions of known pH are in agreement with the values given in the literature. Therefore O.D. measurements lead to pH determination. Variation of O.D. of bromocresol purple vs pH at 549 nm is

given on Fig. 2. The pH measurement range of one indicator is quite narrow, but the use of the three vital indicators allows to scan the scale of pH values observed in the rhizosphere.
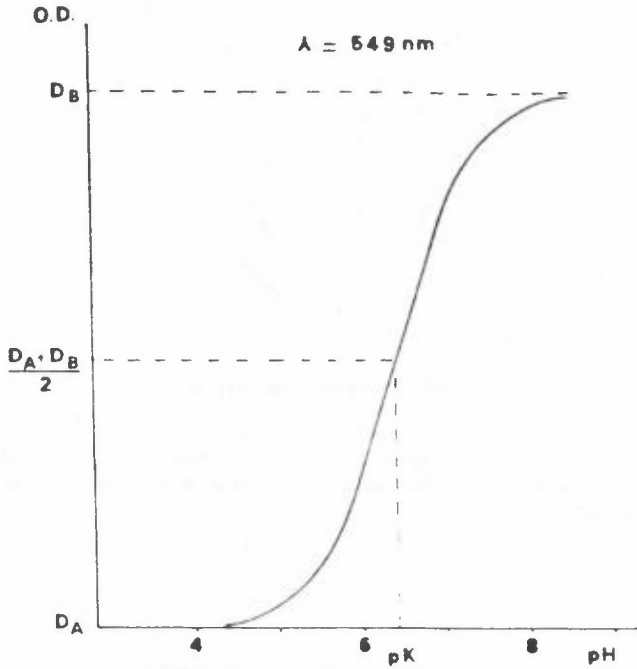

Figure 2. Variation of O.D. of bromocresol purple versus pH.

This relation is used to measure pH gradients around a root of rape plantlet (*Brassica napus*), precultivated in water during three days, and embedded in an agar medium containing the pH indicator. Optical densities are determined by spectrodensitometric scanning perpendicularly to the root axis.

Results

An illustration of the possibilities of the technique is given on Fig. 3: relatively weak pH changes around the root can be accurately measured, and evolution of pH gradients can be followed with time. The precision in pH measurement is about 0.05 units and spatial resolution is 0.4 mm.

Furthermore the realization of successive scannings along the root make possible the establishment of pH spatial distribution and accordingly the localization of the most active root zone for proton exsorption. As an example, Fig. 4 shows pH
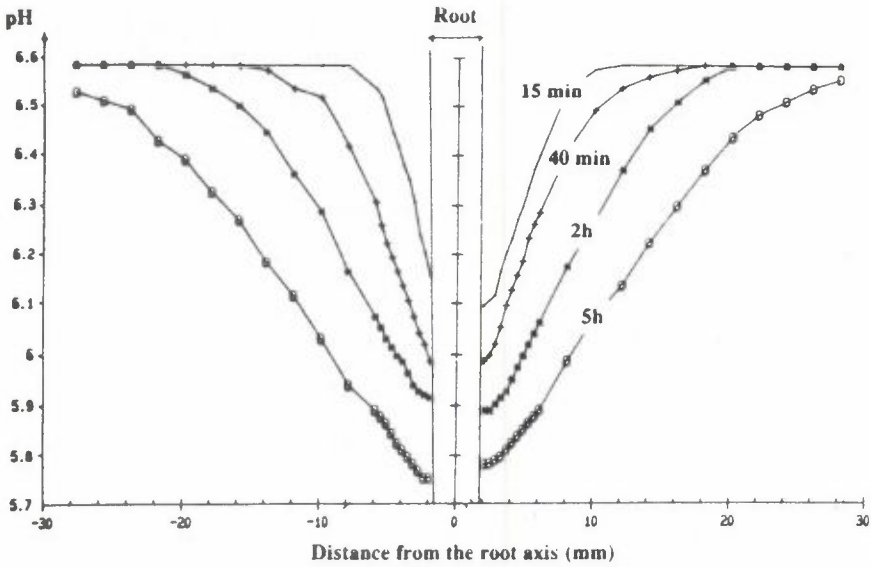
Figure 3. pH gradient around the subapical zone of a root and its variation with time (root of *Brassica napus* precultivated during 4 days in water; bromocresol purple concentration: 60mg/l; initial pH of agar gel:6.6).
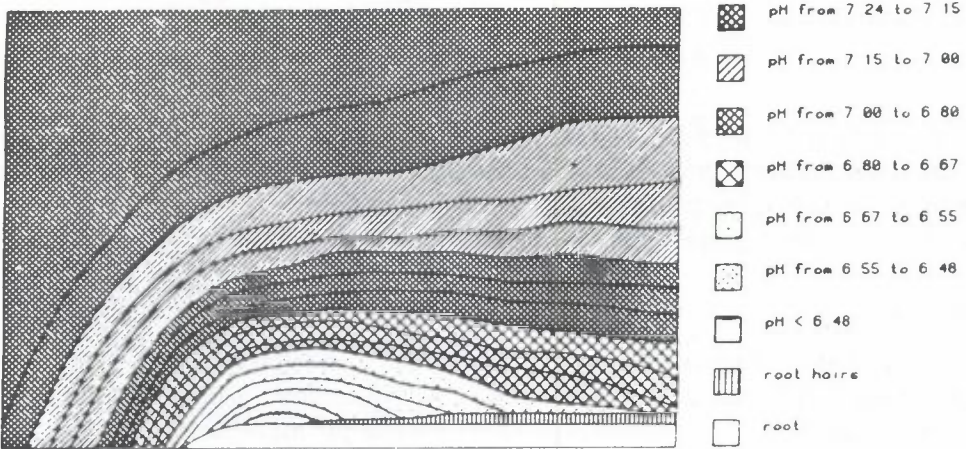

Figure 4. pH gradients distribution along a root of *Brassica napus* showing the greater activity of subapical zone. (Experimental conditions are similar to the preceding experience with a time of contact root-agar = 1 hour).

isovalues curves around a collateral root. The greatest acidification is observed near the subapical zone.

Conclusion

In conclusion this method gives accurate measures of pH with a suitable spatial resolution. Its non-destructive character permits repeated measures with time. Therefore the method would be convenient for studying proton fluxes. Its main disadvantage lies in the necessity of using a transparent medium like agar gel.

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