Plant and Soil **154**: 151–159, 1993.

PLSO 9873

Combined use of colorimetric and microelectrode methods for evaluating rhizosphere pH*

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Received 8 December 1992. Accepted in revised form 7 May 1993

Key words: aseptic, bromocresol purple, genotypic screening, minirhizotron, rhizoplane, rhizosphere-acidification, root zones

Abstract

Plant control of rhizosphere pH is important for nutrient mobilization and uptake, and also affects microbial activity and pathogens in the vicinity of the root. Limited information is available on the ability of plant species and genotypes within a species to induce pH changes in the rhizosphere. A growth chamber study was conducted to characterize patterns of pH change within the rhizosphere of selected genotypes in an alkaline environment with a balanced nutrient supply. After germination in incubators, seedlings of 32 genotypes of maize (*Zea mays* L.), soybean (*Glycine max.* L.), sorghum (*Sorghum bicolor* L.), sordan [sorghum (*Sorghum bicolor* L.), sudangrass (*Sorghum sudanese* L.) hybrid], wheat (*Triticum aestivum* L.), oats (*Avena sativa* L.), and barley (*Hordeum vulgare* L.) were transferred into aseptic agar medium (pH 7.6) with bromocresol purple indicator. Ability of the embedded roots to induce rhizosphere pH change was followed by photographing the color change of the bromocresol purple indicator. The pH for selected genotypes at different root zones (maturation, elongation, meristematic) was also monitored by a microelectrode at 1-, 2-, 3- and 4-mm distances from the root surface. Rhizosphere acidification for selected genotypes within a species were in the order: soybean, Hawkeye > PI-54169; maize, Pioneer-3737 > Pioneer-3732 > CM-37; sordan, S-757 > S-333; sorghum, SC-33-8-9EY ≃ SC-118-15E; barley, Bowman > Primus II; oats, Hytest > SD-84104. The pH patterns within the root system varied from species to species. The highest amount of acidification was found at the elongation and meristematic zones for soybean, while the highest amount of acidification was found at the maturation zone for barley under the same experimental conditions. The agar method allowed the determination of a genotype's capability to induce rhizosphere pH changes while the microelectrode method is necessary for quantifying the spatial variation of specific root developmental zones with high resolution.

Introduction

Root induced pH change in the rhizosphere (soil-root interface) is a known phenomenon (Marschner et al., 1986; Nye, 1981), and has important consequences for nutrient solubility and uptake. However, very few detailed studies have measured rhizosphere pH for different species and genotypes (Römheld and Marschner, 1984). Progress in such studies is restricted by the lack of convenient methods to monitor microsites at the rhizosphere. The rhizosphere pH values for some species have not only revealed large differences between plant species (Marschner and Römheld, 1983; Schaller and

*This work is a part of H.T. Gollany's dissertation in partial fulfillment of the requirements for the PhD degree.

Fischer, 1985; Sarong et al., 1989), but also in genotypes within a species (Clark and Brown, 1974; Römheld and Marschner, 1984). Differences in rhizosphere pH patterns of different zones along a root have also been observed (Häussling et al., 1985; Römheld and Marschner, 1984). Within the root system of the same plant, differences in rhizosphere pH values among root tip, cell division zone, and cell elongation zone can be observed (Blanchar and Lipton, 1986; Häussling et al., 1985; Marschner and Römheld, 1983; Pilet et al., 1983). Schaller and Fischer (1985) found that rhizosphere pH values of maize at the root tip and root hair zone differed by 4 units.

Root induced pH changes in the rhizosphere depend on soil buffering capacity and nitrogen source for some species. Fertilization of soybeans with NH_4^+–N decreased rhizocylinder pH (Riley and Barber, 1971). The difference in rhizosphere pH between treatments was as large as 1.9 pH units with an initial pH of 5.2 and as small as 0.2 units when the soil pH was 7.8 prior to N application. Compared to the bulk soil (pH 6.0), the pH in the rhizosphere of maize increased to about 7.5 with 16.6 mg NO_3^-–N/100 g soil and decreased to about 4 with 16.6 mg NH_4^+–N/100 g soil (Marschner and Römheld, 1983). In contrast to maize, the chickpea (*Cicer arenatherum* L.) rhizosphere pH decreased to about 4.5 even when supplied with 8.3 mg NO_3^-–N/100 g soil (Marschner and Römheld, 1983).

Studies of the rhizosphere pH patterns for crops under balanced nutrient supply are few compared with the number of those under nutrient stress (Marschner and Römheld, 1983; Moorby et al., 1988; Römheld and Marschner, 1984). Also, all the rhizosphere pH pattern studies have been carried out in an acidic environment (Blanchar and Lipton, 1986; Pijnenborg et al., 1990; Schaller and Fischer, 1985).

Changes in pH along roots of intact plants can be demonstrated after embedding the roots in agar mixed with a pH indicator (Weisenseel et al., 1979). Marschner et al. (1982) developed a simple method to measure pH changes based on this idea. The objective of this study was to characterize patterns of pH change within the rhizosphere of selected genotypes in an alkaline environment with balanced nutrient supply.

Materials and methods

Seed sterilization and pre-germination conditions

Seeds of maize (*Zea mays* L., cv., SD 38, A654, W153R, CM37 × W153R, CM-37, WF-9, Pioneer-3732 and -3737)[1]; soybean (*Glycine max*. L., cv., PI-54619 and Hawkeye)[1]; sorghum (*Sorghum bicolor* L., cv., SC-33-8-9EY, and SC-118-15E); sordan [sorghum (*Sorghum bicolor* L.), sudangrass (*Sorghum sudanese* L.) hybrid, cv., S-333, and S-757)[1]; wheat (*Triticum aestivum* L., cv., Marshall, Stoa, Prospect, Guard, Len, and Butte 86)[1]; oats (*Avena sativa* L., cv., Lancer, Starter, Kelly, Settler, SD-84104 and Hytest)[1]; and barley (*Hordeum vulgare* L., cv., Primus II, NEF-Azure, Morex, Robust, Glenn and Bowman)[1] were surface-sterilized in 10% NaOCl for 10 minutes under a sterilization hood. Seeds were thoroughly rinsed with sterile deionized water and transferred after 5 minutes to a saturated solution of $CaSO_4$.

Filter papers (7.0 cm Whatman no. 42)[1] were cut into four pieces and moistened with saturated $CaSO_4$. Each seed was sown on a piece of filter paper and transferred into a sterilized plastic pipet tip (no. 6241-10, Cole-Parmer Instrument Co. Chicago, IL)[1] after cutting 2- to 3-cm from the narrow end of the tip. The pipet tips were used to prevent the organic exudate from contacting the agar and to isolate the agar and roots from contamination by atmospheric microorganisms. Individual tips were laid, 2 cm apart, on wet germination paper towels, wrapped and placed in a plastic bag, allowing germinated seedlings to emerge in a moist environment.

The bags containing the seeds were placed in plastic beakers and transferred from the laminar flow hood to incubators. Incubator conditions were as follows: wheat, barley and oats were grown at 20°C and 8 hours of light daily; maize and soybeans at 25°C and 8 hours light; and sorghum and sordan at 30°C and 8 hours light. After germination, seedlings were removed from

[1]This paper reports the results of research only. Mention of a trademark, proprietary product, or vendor does not constitute a guarantee or warranty of the product by the South Dakota Agricultural Experiment Station, and does not imply their approval to the exclusion of other products or vendors that may also be suitable.

the incubators. The roots were rinsed with deionized water under the sterilization hood and transferred to minirhizotrons for embedding in the agar solution.

Minirhizotron preparation

Minirhizotrons were constructed from 100 × 15 mm sterilized plastic petri dishes. A hole (port) drilled in the edge of each dish allowed the root, protruding from each pipet tip, to enter the petri dish (Fig. 1).

Agar preparation

An aseptic orchid agar (Bacto orchid agar, Difco Laboratories, Detroit, MI)[1] was substituted for unsupplemented unsterilized acidic-agar as a modification of the Marschner et al. (1982) method. Deionized water, 950 mL, was added to 0.09 g bromocresol purple with 37 g of orchid agar [containing 1.0 g $Ca(NO_3)_2$, 0.5 g $(NH_4)_2SO_4$, 0.25 g $MgSO_4$, 0.25 g KH_2PO_4, 0.025 g $FeSO_4$, 0.0075 g $MnSO_4$, and 20 g Saccharose]. Bromocresol purple is purple at pH 6.8 and becomes yellow at pH 5.2 (Clark, 1928). The pH was adjusted to 7.6 with 0.1 m*M* NaOH. Deionized water was added to the 1000 ml mark, and the mixture was sterilized in an autoclave. The liquid agar was removed from the autoclave after 45 minutes, and a mixture of 10 mg phenol, 5 mg Benlate [methyl-1-(butylcarbamoyl)-2-benzimidazolecarbamate], and 5 mg Mancozeb 200 DF (manganese ethylenebisdithiocarbamate) was added. The pH of the agar was then readjusted to 7.6.

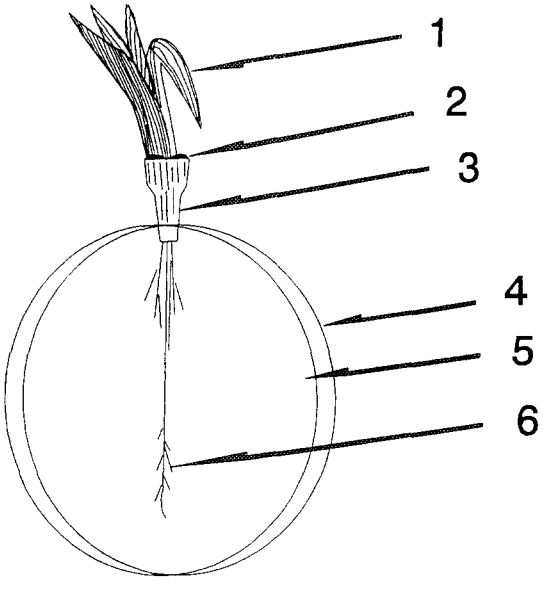

Fig. 1. Diagram of the minirhizotron used to grow seedlings. 1. Seedling; 2. Filter paper; 3. Pipet tip; 4. Plastic petri dish; 5. Agar; 6. Root.

Embedding of the roots

After 4 days (maize, sorghum, sordan and soybeans) or 6 days (wheat, barley and oats) of germination, seedlings were carefully transferred to the petri dishes under the sterilization hood. The roots were inserted through preformed ports, oriented with a sterilized glass rod, and the molten agar medium (38–40°C) was poured into the petri dishes. The agar gelled rapidly when cooled to 25°C. A second application of molten agar was applied, if necessary, to ensure complete embedding. Lids were replaced and the dishes were sealed with parafilm to minimize evaporative losses. Aluminum foil, wrapped around each dish, protected the roots from exposure to direct light.

The petri dishes were transferred to a growth chamber and positioned at a 45° angle. The seedlings were kept on a night/day regime of 8/16 hours, 18–25°C, relative humidity 65–75%, and photon flux density (400–700 nm) of 460 μmol m^{-2} s^{-1}. The roots continued to grow in the agar medium for several days, although the growth rate was lower than in a soil environment. Three petri dishes without plants were maintained at the same conditions to serve as controls (bulk agar). Experiments were carried out two times independently with two or three replicates. The results presented are representative of at least four individual plants.

pH determination

Photographs were taken after 24–48 hrs. Color patterns, characteristic of each genotype or species, appeared next to the roots. The rhizosphere pH along individual roots was determined by comparison with calibration standards of bromcresol purple in agar. The ability of the

genotype to induce pH change in the rhizosphere was estimated by rating the color after embedding for 24 hours in the agar as follows: 1 = Slight, 5–10% yellow; 2 = Moderate, 10–40% yellow; 3 = Strong, 40–80% yellow. Two genotypes within each species were selected for quantifying the pH changes with a microelectrode, one with slight and one with strong ability to induce rhizosphere pH changes as indicated by the agar technique.

Evaluation of the agar technique

In subsequent experiments, the aseptic agar technique was repeated on the selected genotypes of maize, sorghum, sordan, oats and barley. Following observations of the pH changes in the agar, the rhizosphere pH was monitored with a pH-glass microelectrode (Conkling and Blanchar, 1989) at selected intervals after planting (36, 48, or 72 hours). The pH measurements at different developmental zones along a root (maturation, elongation, and meristematic) were taken at 1-, 2-, 3-, and 4-mm distances from the root surface.

Results and discussion

Ability of genotypes to induce rhizosphere pH change

The aseptic agar technique gave an overall picture of the behavior of the selected genotypes. The criteria for selection of the genotypes reflected a desire to test many of those in common usage in this region, as well as to examine genotypes with potential rhizosphere acidification as cited in the literature (Römheld and Marschner, 1984; Robbins, 1986). The color of the indicator along the root of soybean cv., Hawkeye changed from violet to orange-yellow. A comparison with the pH calibration standards illustrated a pH change from 7.6 (violet) to 5.5 (yellow-orange) at the rhizoplane (< 1 mm distance from the root surface) within 36 h. Orange-yellow zones reflected the acidification along the root by up to 2.5 units compared to the bulk medium or control. This response was similar to that observed by Römheld and Marschner (1984) for the roots of Hawkeye soybean genotype in non-sterile soils. The agar medium was sterilized and no bacteria or fungal colonies were observed. Nitrogen supply consisted of 0.012 *M* NO_3^- as $Ca(NO_3)_2$ and 0.008 *M* NH_4^+ as $(NH_4)_2SO_4$, therefore the ratio of NO_3^-/NH_4^+ was 1.58. The presence of NH_4^+ could have some effect on the net acidification around the root. The combined effects of an alkaline uptake pattern (Bekele et al., 1983), NO_3^- reduction occurring primarily in shoot (Keltjens, 1982) and H^+ excretion associated with cell division and cell elongation (Pilet et al., 1983; Weisenseel et al., 1979) are possible explanations for these pH changes along the root. The efflux of organic acids appears to have a minor role in maize seedlings, accounting for only 0.2 to 0.3% of acidification (Petersen and Böttger, 1991).

At the same nutrient supply, differences in rhizosphere acidification were observed between plant species. A less distinct color change was observed for sordan species than for soybean species. The differences in rhizosphere acidification patterns between the two plant species may be due to a higher rate of anion uptake (NO_3^-) by the gramineous species (grasses) and lower cation uptake (Ca^{2+}, K^+, ..., NH_4^+) than the dicotyledonous species (Bekele et al., 1983).

Genotypic differences in rhizosphere pH were found not only between different plant species but also between genotypes of the same species. The color rating revealed differences of six wheat genotypes in capability to induce rhizosphere acidification (Table 1). The extent of change in color of pH indicator (from violet to orange-yellow) differed from 1 mm to 10 mm among the genotypes. The presence of NH_4^+ could have contributed to net acidification. Nevertheless, the rhizosphere acidification around the root reflects genotypic differences between the plant species and genotypes within the species. Distinct differences in rhizosphere pH between genotypes probably relate to the differences in the cation/anion uptake rate, root exudation, rate of nitrate reduction, and root respiration patterns (Häussling et al., 1985; Marschner and Römheld, 1983). Similar differences in rhizosphere pH for genotypes within oat, barley, soybean, and maize were observed (Table 1). Significant genotypic variation in

Table 1. Estimated ability to induce pH change in the rhizosphere of various genotypes within plant species, pH change detected by the bromocresol purple agar technique

Plant species	Genotypes	Rhizosphere pH change[a]		
		Slight	Moderate	Strong
Zea mays L.	SD38	•		
	CM-37		•	
	W153R		•	
	A 654		•+[b]	
	Pioneer-3732		•+	
	Pioneer-3737			•+
	W153R × CM-37			•+
	WF-9			•+
Glycine max. L.	PI-54619		•	
	Hawkeye			•+
Sorghum bicolor L.	SC-3-8-9EY		•	
	SC-118-15E		•	
Sorghum bicolor L., X *Sorghum sudanese* L. hybrid	S-333		•	
	S-757		•+	
Triticum aestivum L.	Marshall	•		
	Prospect	•		
	Guard	•+		
	Stoa		•	
	Butte 86			•
	Len			•+
Avena sativa L.	Kelly	•		
	Lancer	•		
	SD 84104	•+		
	Settler	•+		
	Starter		•	
	Hytest		•+	
Hordeum vulgare L.	Robust	•		
	Primus II	•+		
	NEF-Azure		•	
	Morex		•	
	Glenn			•
	Bowman			•+

[a] Slight = 5–10% yellow, moderate = 10–40% yellow and strong = 40–80% yellow after embedding for 24 hrs.
[b] A plus (+) indicates that the color change was closer to the higher end of the scale.

rhizosphere acidification capability was found for maize in the agar. The maize cv. WF-9 had the strongest acidification ability while SD38 had the weakest acidification ability among the genotypes of maize.

Nutrients of low solubility (P, Fe, Zn, Mn) in the bulk soil could be solubilized in the rhizosphere and become available to those genotypes with strong acidification capability particularly in a calcareous (alkaline) soil. The P content of soybeans was closely correlated to the pH of the rhizocylinder (Riley and Barber, 1971). Root induced pH changes in the rhizosphere of rape (*Brassica napus* L.) resulted in more P removed from the rhizosphere than could be explained, using a 0.5 M $NaHCO_3$ solution commonly used for soil test extractions (Gahoonia and Nielsen, 1992). Sarkar and Wyn Jones (1982) reported that the shoot Fe, Mn and Zn content were significantly correlated with the extractable

rhizosphere Fe, Mn, and Zn and inversely proportional to rhizosphere pH. Enhanced MnO_2 reduction to Mn^{2+} was also observed by Marschner and coworkers (1982) around the apical zones of Fe-deficient sunflower root due to rhizosphere acidification. Two maize genotypes (WF-9 and ys 1/ys 1) with wide differences in Fe uptake and utilization, differed in their susceptibility to Fe deficiency (Clark and Brown, 1974). Iron-stressed WF-9 produced higher amounts of H^+ and 'reductant' in nutrient solutions and reduced more Fe at the root surface than the ys 1/ys 1 genotype (Clark and Brown, 1974). Römheld and Marschner (1984) studied the pH changes in the rhizosphere of the same genotypes with the aid of antimony microelectrodes. They found that the rhizosphere pH (6.8) was higher in the 'Fe-inefficient' genotype ys 1/ys 1 compared to (5.6) in the 'Fe-efficient' genotype WF-9. This is in agreement with our finding that the WF-9 genotype had the greatest capability to acidify the rhizosphere of the eight genotypes tested.

Genotypic differences in rhizosphere acidification not only could modify nutrient availability and influence uptake, but also could affect microbial activity and pathogen population. Take-all disease of wheat was found to be correlated with the rhizosphere pH (Smiley and Cook, 1973; Smiley, 1974).

pH patterns within the root system

The pH measurement with a microelectrode for selected genotypes quantified the pH changes revealed by the color indicator by detecting a spatial pH gradient along specific root developmental zones with high resolution. The acidification within the root system varied from species to species (Table 2). The pH decreased to 4.82 and 4.95 in the rhizosphere around elongation and meristematic zones, respectively, compared to the control (pH = 7.6) without plants (Fig. 2). The soybean cv. Hawkeye decreased the pH by 2.78 and 2.65 units relative to the control in the elongation and meristematic zones, respectively. The pH reduction was more pronounced at the

Table 2. A comparison of root zones pH pattern 72 hrs after embedding roots in the aseptic agar medium. Mean rhizosphere pH measured by microelectrodes over 4-mm distance from root surface

Plant species	Genotype	pH (n = 16)		
		Maturation Zone	Elongation Zone	Meristematic Zone
Control		7.58[a](0.28)[b]		
Soybean	Hawkeye	6.28 (0.18)	4.82 (0.16)	4.95 (0.10)
	PI-54169	6.53 (0.17)	6.30 (0.13)	5.75 (0.12)
Maize	Pioneer-3737	5.72 (0.19)	5.23 (0.36)	6.41 (0.26)
	Pioneer-3732	6.23 (0.25)	6.02 (0.22)	5.23 (0.39)
	CM-37	5.95 (0.19)	6.41 (0.22)	6.51 (0.21)
Sorghum	SC-33-8-9EY	7.18 (0.08)	6.38 (0.01)	6.30 (0.02)
	SC-118-15E	6.86 (0.09)	6.83 (0.06)	6.62 (0.07)
Sordan	S-757	5.97 (0.04)	6.43 (0.01)	6.10 (0.02)
	S-333	6.79 (0.13)	6.46 (0.04)	6.13 (0.03)
Oats	Hytest	6.89 (0.05)	7.08 (0.13)	5.99 (0.04)
	SD 84104	7.56 (0.09)	7.45 (0.17)	6.18 (0.01)
Barley	Bowman	6.20 (0.08)	6.78 (0.02)	7.58 (0.07)
	Primus II	6.01 (0.04)	7.68 (0.03)	7.80 (0.01)

[a] Mean value (n = 7) measured with pH microelectrode in sterilized bromocresol agar.
[b] Standard error of the mean.

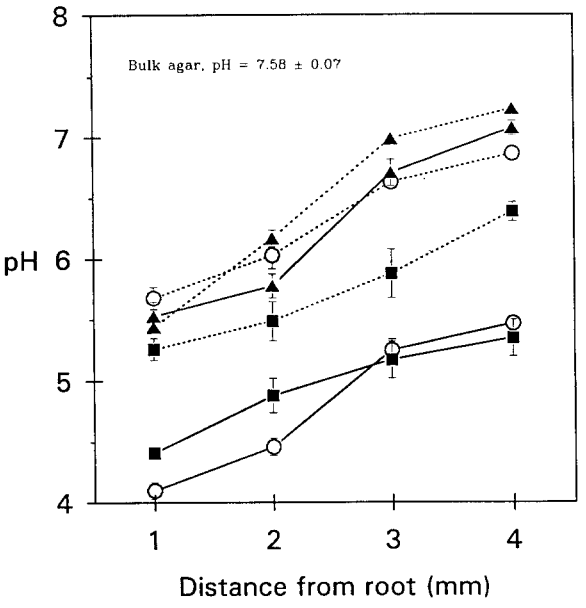

Fig. 2. pH changes measured with microelectrode at the three root zones (maturation, ▲; elongation, ○; meristematic, ■) within the root system of Hawkeye (——) and PI-54169 (···) soybean genotypes after embedding for 72 h in an agar medium with bromocresol purple. Bars = ± Standard error of the mean (n = 4).

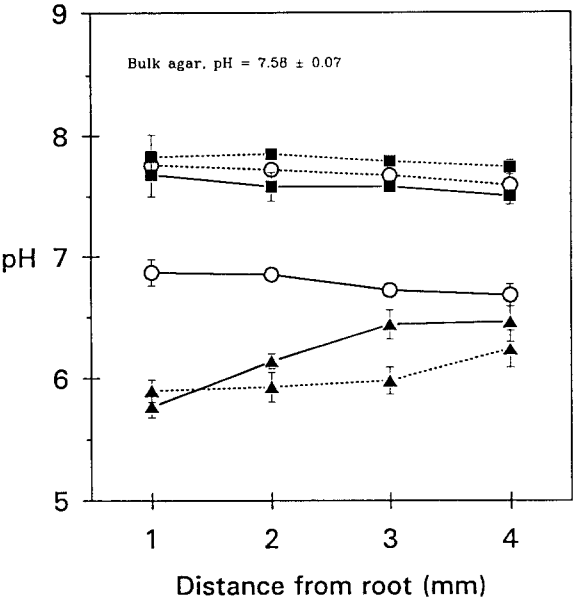

Fig. 3. pH changes measured with microelectrode at the three root zones (maturation, ▲; elongation, ○; meristematic ■) within the root system of Bowman (——) and Primus II (···) barley genotypes after embedding for 72 h in an agar medium with bromocresol purple. Bars = ± Standard error of the mean (n = 4).

three root developmental zones of Hawkeye compared to PI-54169.

Root induced acidification was also observed for maize, sorghum, sordan, and oats (Table 2). The lower pH in the rhizosphere of apical zones has been shown to be related to enhanced H^+ secretion from roots in connection with cell extension (Pilet et al., 1983; Weisenseel et al., 1979).

In contrast, pH reduction for barley was found primarily in the maturation zone (Fig. 3). The Primus II barley genotype did not alter the rhizosphere pH in the meristematic or elongation zones compared to the control. There was also no pH change in the meristematic zone for the Bowman genotype.

The pH difference measured with the microelectrode, between root developmental zones, within the root system of Primus II (1.79 units) was more pronounced than that of Bowman (1.38 units) or any other genotypes within other species. The reason for the pH differences within a root system is not fully understood. Possible explanations for the difference may be due to the cation/anion uptake rate at specific root zones and the physiological characteristics of each genotype. The difference in rhizosphere pH between various root zones is presumably due to differences in metabolism along the root axes (Marschner and Römheld, 1983). Localized acidification of the rhizosphere could be of significant ecological importance for some plants as it increases organic exudate degradation and mobilizes sparingly soluble P and Fe minerals (Hoffland et al., 1989). Localized differences of pH within the root system could also create specific niches for microorganisms. Bashan and Levanony (1988) reported that the rhizosphere acidification by wheat markedly increased adsorption of bacteria to soil particles. The physicochemical differences that occur within the rhizosphere have implications for the ontogeny of root diseases as well as nutrient uptake. Further work is needed to determine how genotypic differences in rhizosphere acidification correlate with nutrient acquisition efficiency.

Conclusions

The aseptic agar technique allowed visualization of the behavior of the root systems. This technique is suitable for identifying the ability of a genotype to induce rhizosphere pH changes. Change in rhizosphere pH was due to the root activity and appeared not to be due to microbial activity or seed exudates. Genotypes within species under conditions of the study could be separated on the basis of the intensity of pH change within the rhizosphere. Rhizosphere acidification for selected genotypes within a species were in the order: soybean, Hawkeye > PI-54169; maize, Pioneer-3737 > Pioneer-3732 > CM-37; sordan, S-757 > S-333; sorghum, SC-33-8-9EY ≃ SC-118-15E; barley, Bowman > Primus II; oats, Hytest > SD-84104. The resolution of the agar technique was limited due to the difficulty of visually detecting color gradients near the root. The agar technique is not well adapted for determining pH patterns within the root system. However, this technique could be enhanced by electronically evaluating the color, (for example, by use of an image analyzer).

The pH measured with the microelectrode quantified the changes in pH revealed by the color indicator. The microelectrode, however, detected spatial changes of specific root zones with higher resolution than the color indicator. The pH patterns within the root system varied from species to species. The highest amounts of acidification were found at the elongation and meristematic zones for soybean, while the highest amount of acidification in barley rhizosphere was found in the maturation zone. The combined methods of qualitative and quantitative pH measurement of specific root zones allowed the determination of genotypically determined differences in rhizosphere pH between species, between genotypes within the same species and within root zones.

Genotypic difference in rhizosphere acidification capability has implications for effective use of fertilizer, herbicides, crop rotation, interplanting, and companion crops. Such knowledge can potentially be used to identify particular genotypes to exploit specific environments. This would result in higher nutrient availability or less nutrient toxicity in less than ideal soil environments.

Acknowledgements

The author (H.T.G.) is very grateful to Dr R W Blanchar for the opportunity to learn pH-glass microelectrode fabrication in his laboratory at the University of Missouri in Columbia. Thanks also to his staff, especially Dr B L Conkling, for expert technical instruction. Seeds of soybean were kindly supplied by Dr R Nelson, University of Illinois, of sorghum by Drs R Clark and J F Pederson, University of Nebraska, and of maize, wheat, and oats by Drs Z Wicks, F Cholick, and D Reeves, South Dakota State University. Thanks to Drs D D Malo and W Riedell for review and comments regarding this manuscript. Supported in part by Project H-250 of the South Dakota Agricultural Experiment Station (Journal article No. 2695).

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Section editor: R Merckx